FIG. 1

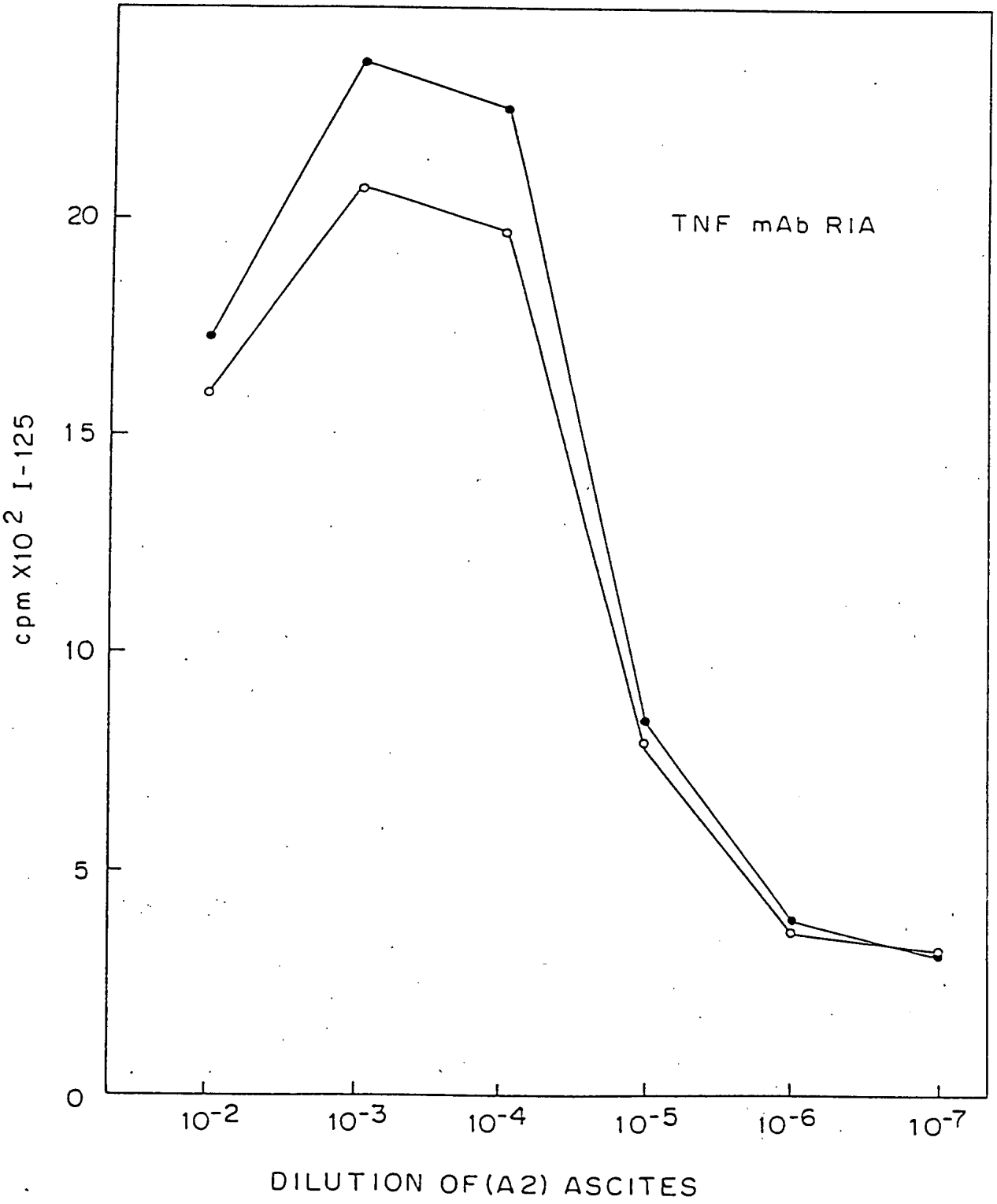

TNF mAb RIA
cpm X10 2 I-125
20
15
10
5
0
10-2
10-3
10-4
10-5
10-6
10-7
DILUTION OF (A2) ASCITES

FIG. 2

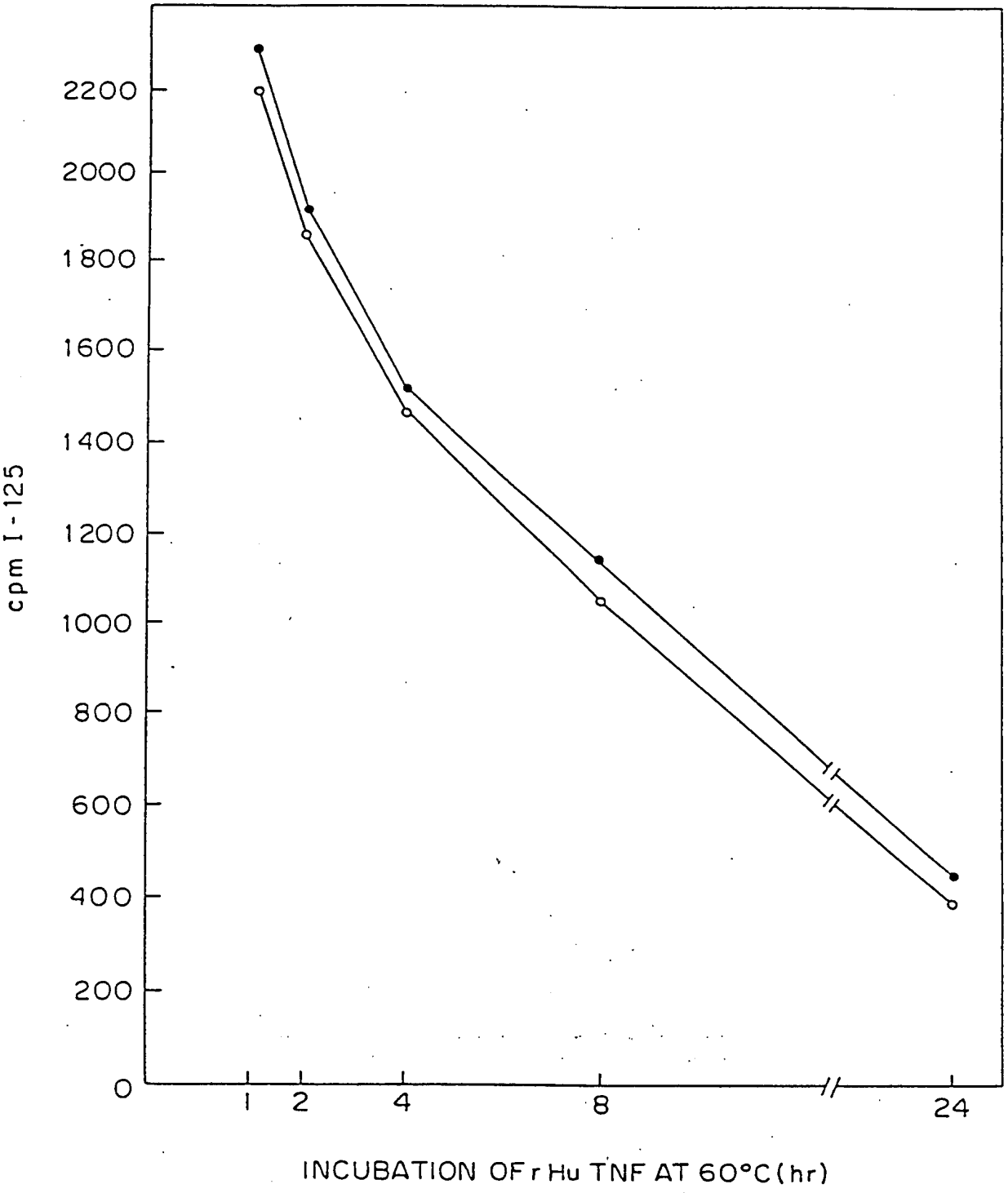

2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
cpm I-125
1
2
4
8
24
INCUBATION OF r Hu TNF AT 60°C (hr)

FIG. 3

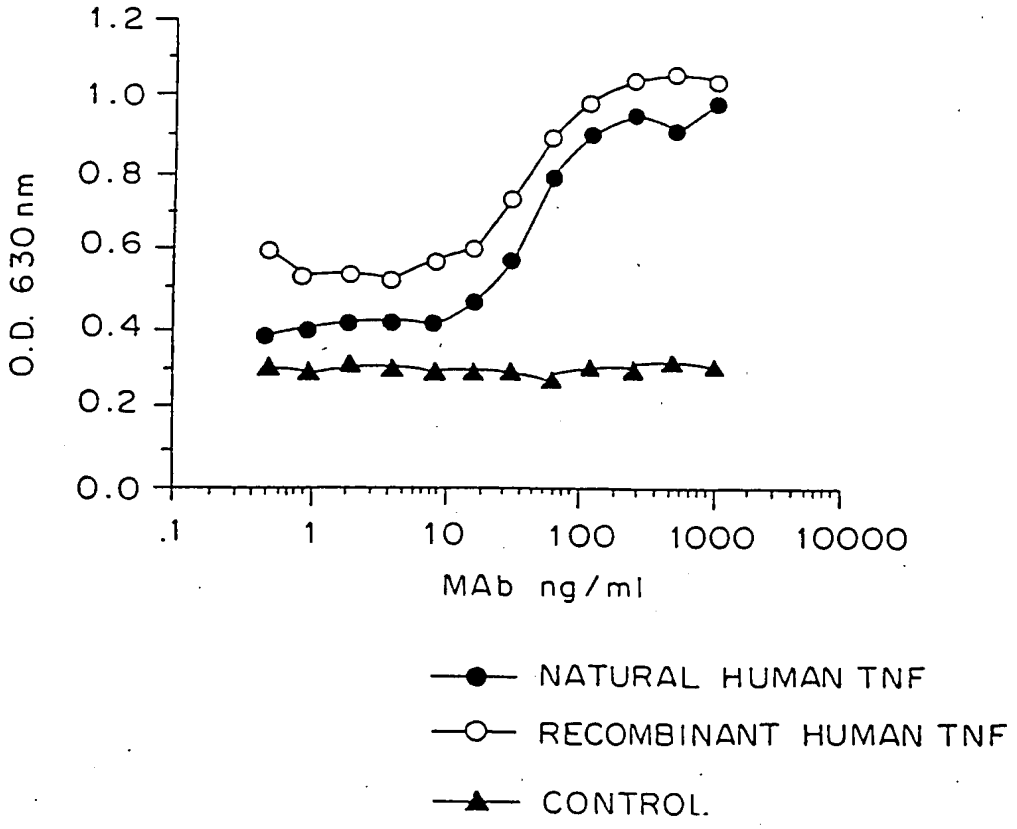

1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D. 630 nm
.1
1
10
100
1000
10000
MAb ng/ml
NATURAL HUMAN TNF
RECOMBINANT HUMAN TNF
CONTROL.

FIG. 4

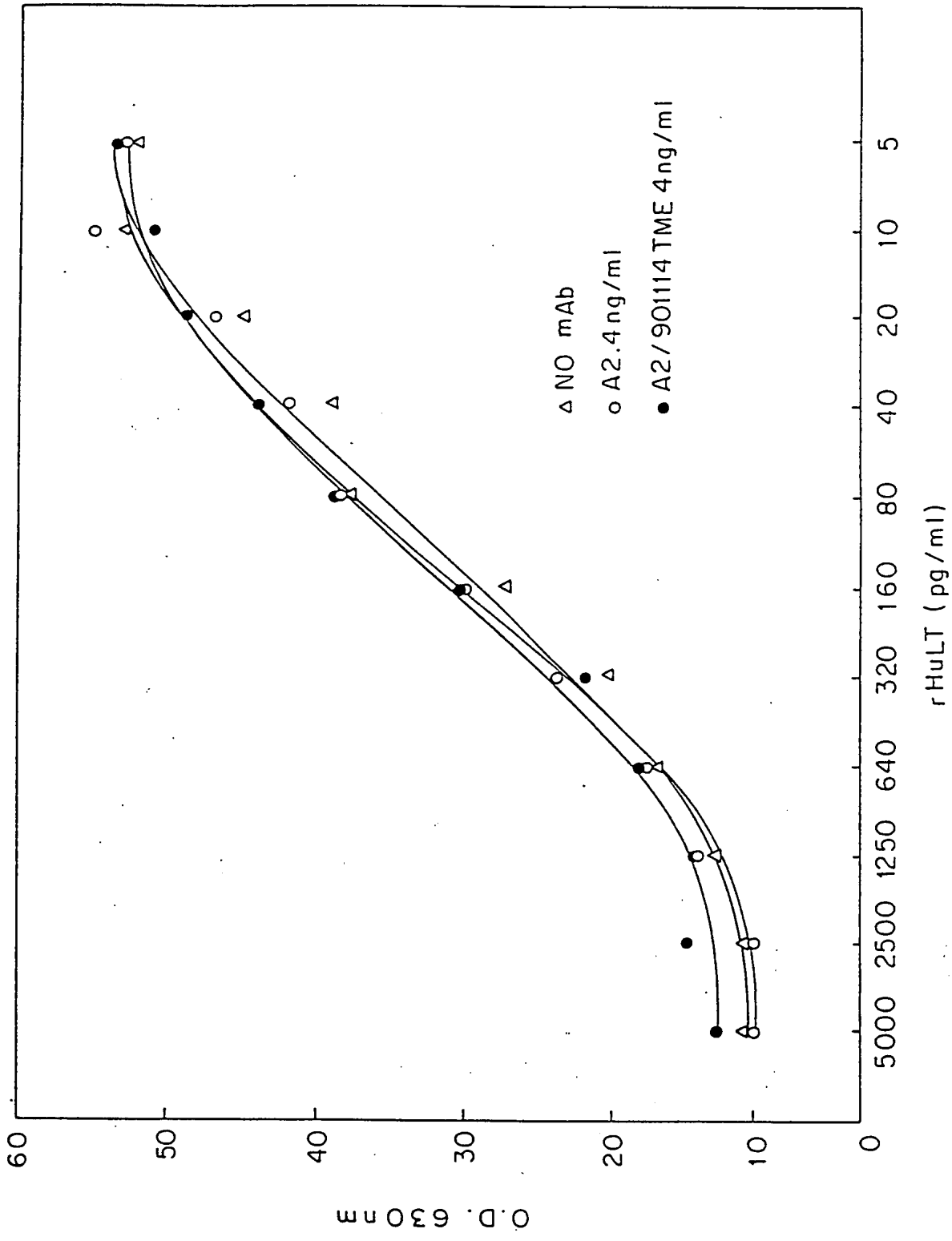

O.D. 630nm
60
50
40
30
20
10
0
5000 2500 1250 640 320 160 80 40 20 10 5
rHuLT (pg/ml)
△ NO mAb
○ A2.4ng/ml
● A2/9O1114 TME 4ng/ml

FIG. 5

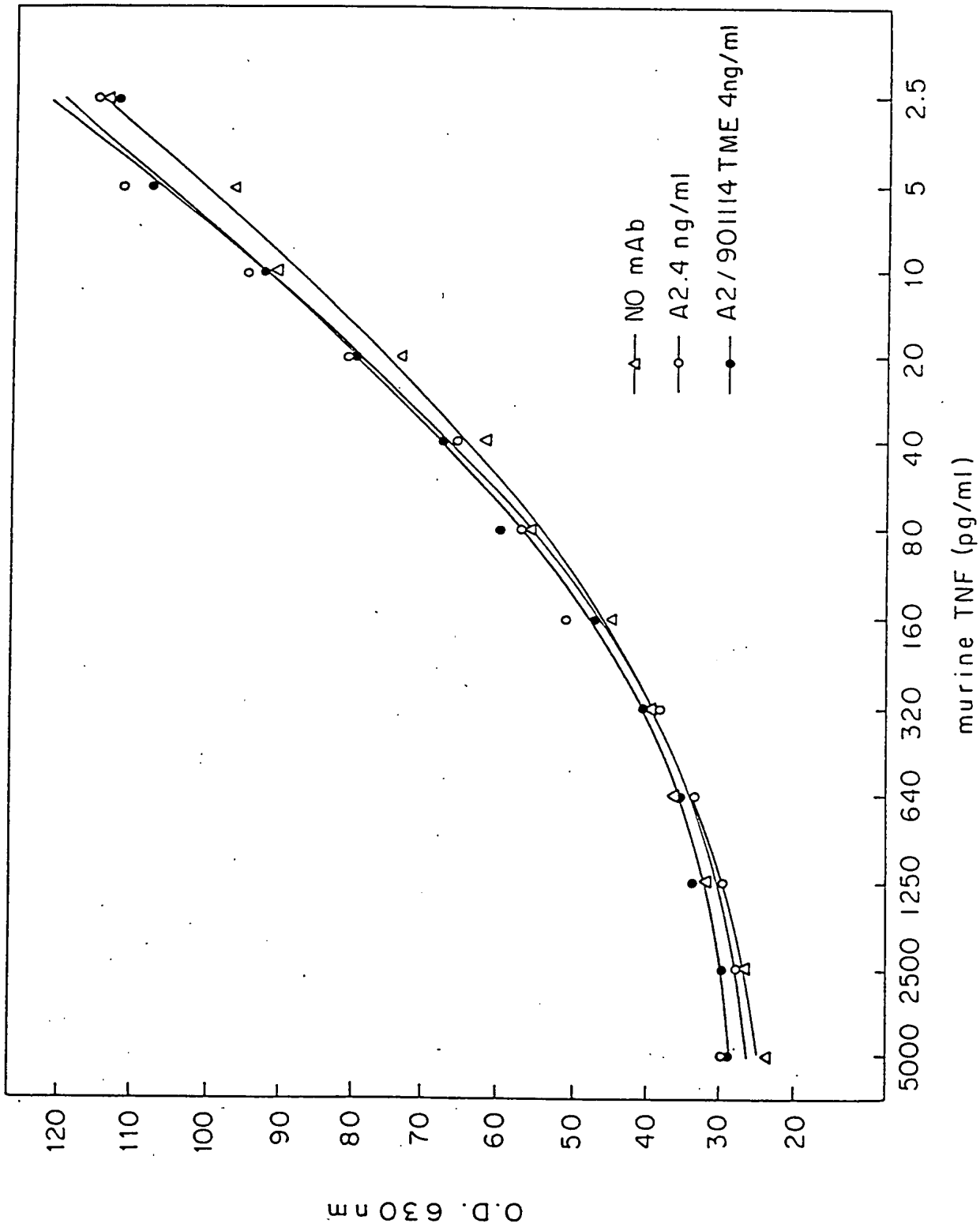

NO mAb
A2.4 ng/ml
A2/9011114 TME 4ng/ml
O.D. 630 nm
murine TNF (pg/ml)
120
110
100
90
80
70
60
50
40
30
20
5000
2500
1250
640
320
160
80
40
20
10
5
2.5

FIG. 6

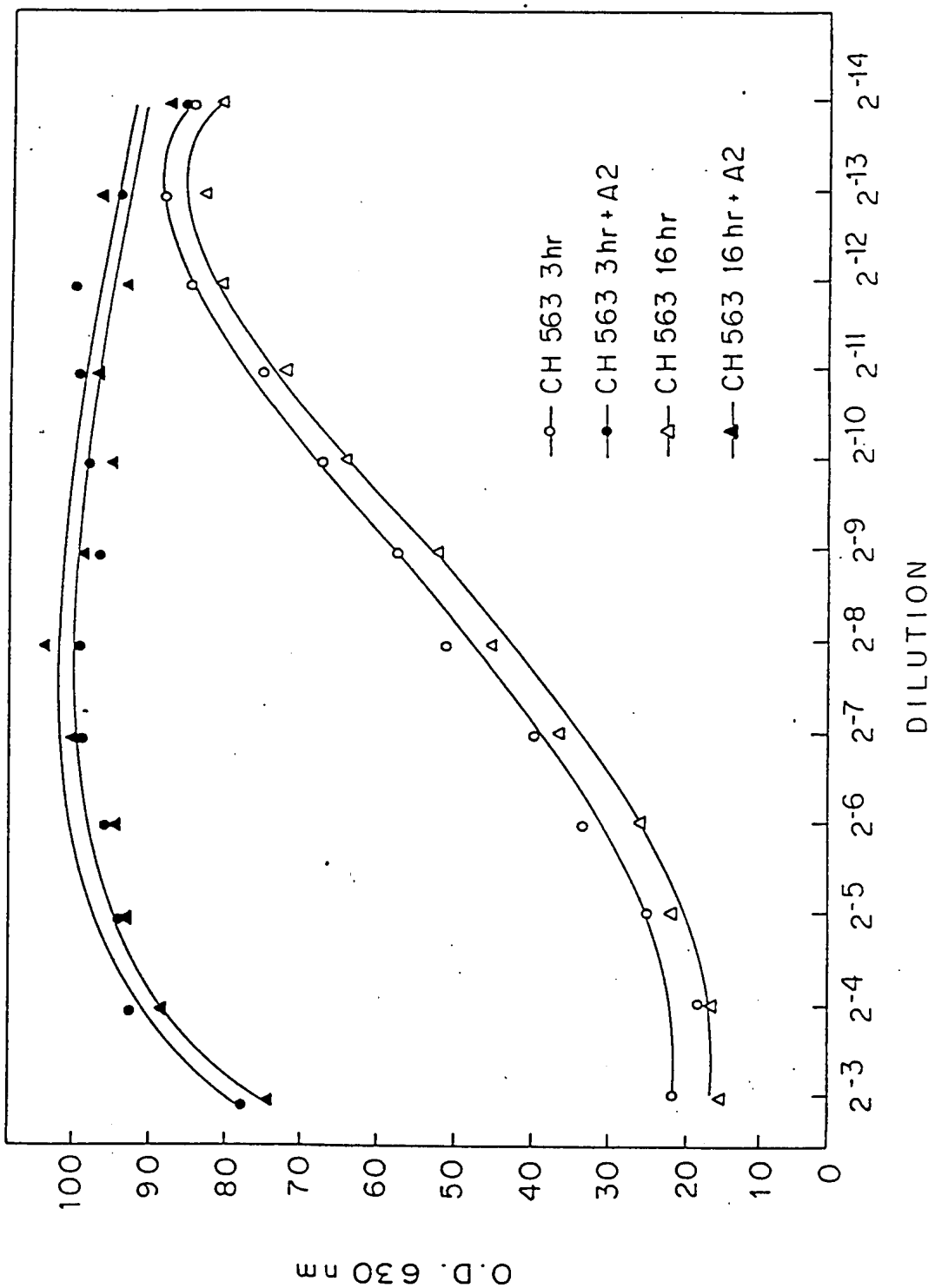

O.D. 630 nm
100
90
80
70
60
50
40
30
20
10
0
2-3
2-4
2-5
2-6
2-7
2-8
2-9
2-10
2-11
2-12
2-13
2-14
DILUTION
CH 563 3hr
CH 563 3hr + A2
CH 563 16hr
CH 563 16hr + A2

FIG. 7

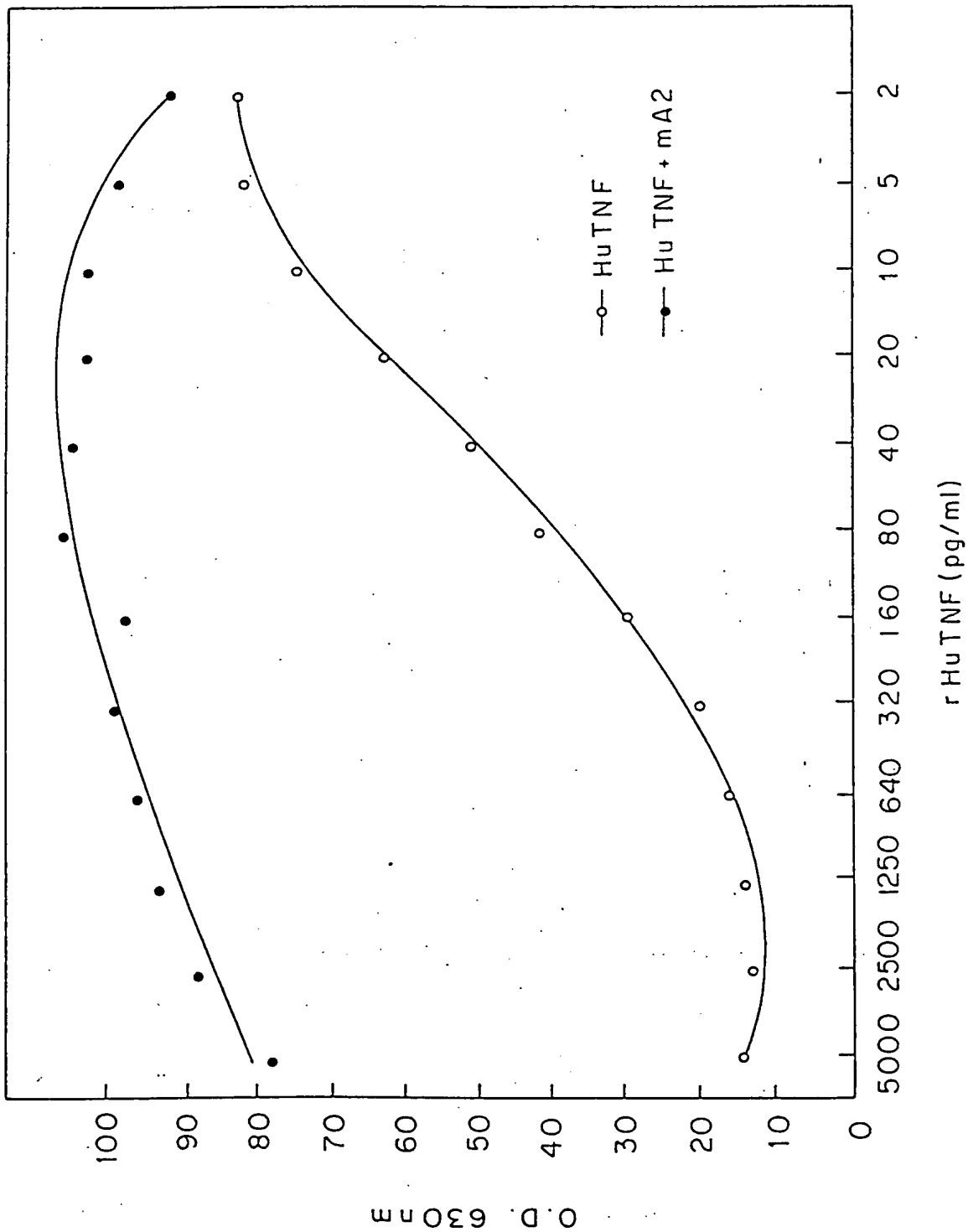
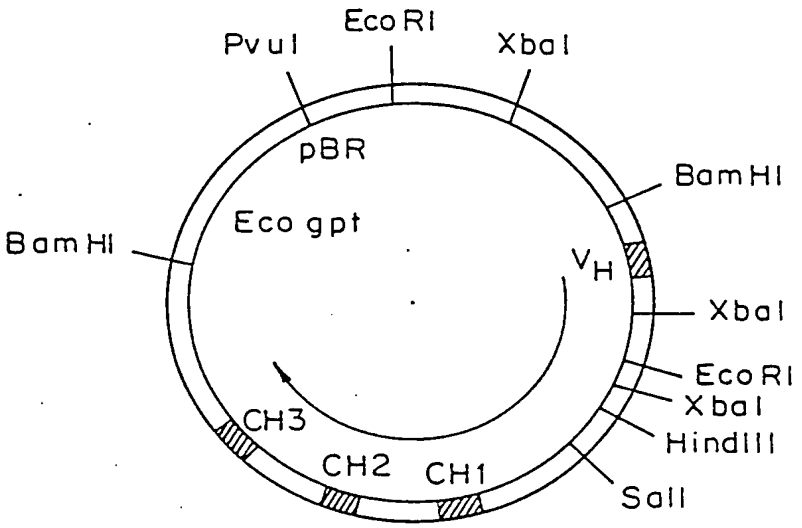
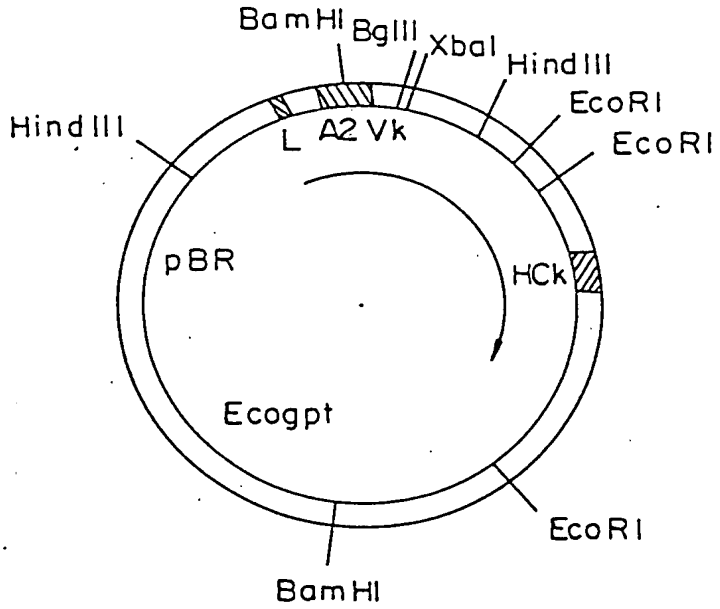

O.D. 630nm
100
90
80
70
60
50
40
30
20
10
0
5000 2500 1250 640 320 160 80 40 20 10 5 2
r Hu TNF (pg/ml)
Hu TNF
Hu TNF + mA2

FIG. 8a



pA2HG1apgpt

FIG. 8b



pA2HuKapgpt

FIG. 9a

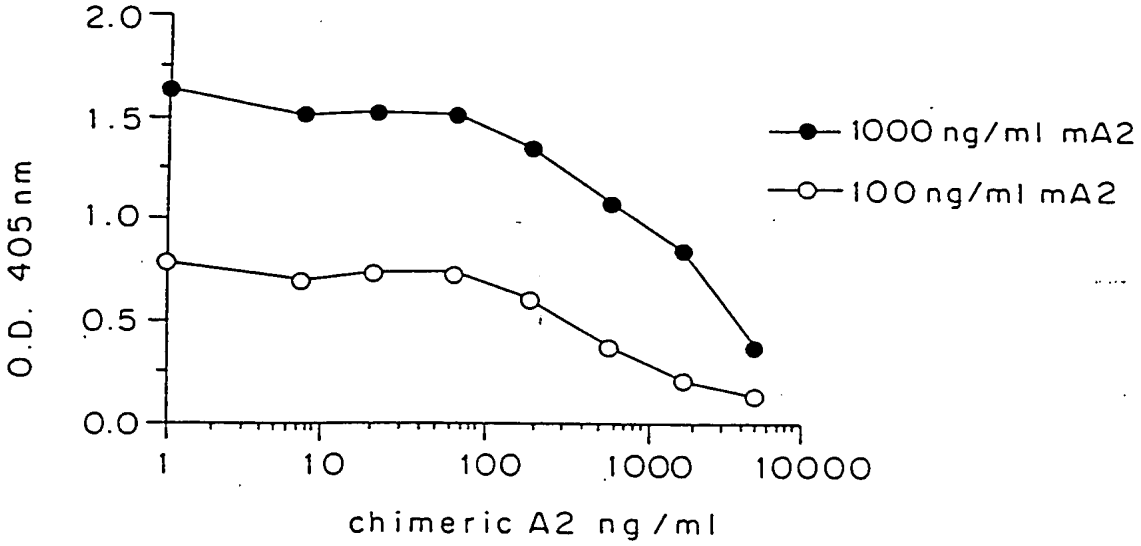

2.0
1.5
1.0
0.5
0.0
O.D. 405nm
1
10
100
1000
10000
chimeric A2 ng/ml
1000 ng/ml mA2
100 ng/ml mA2

FIG. 9b

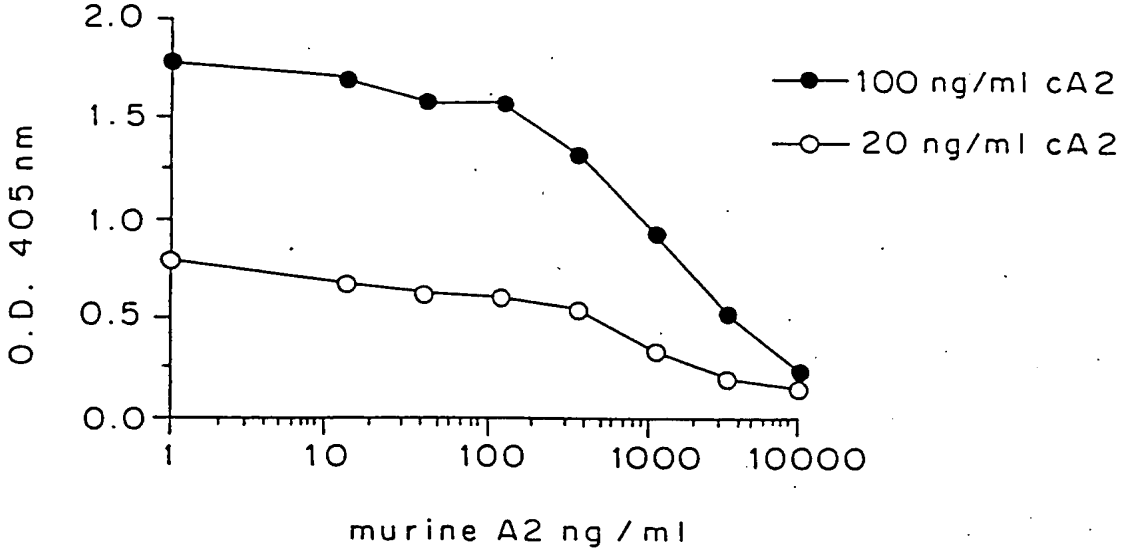

2.0
1.5
1.0
0.5
0.0
O.D. 405nm
1
10
100
1000
10000
murine A2 ng/ml
100 ng/ml cA2
20 ng/ml cA2

FIG. 10a

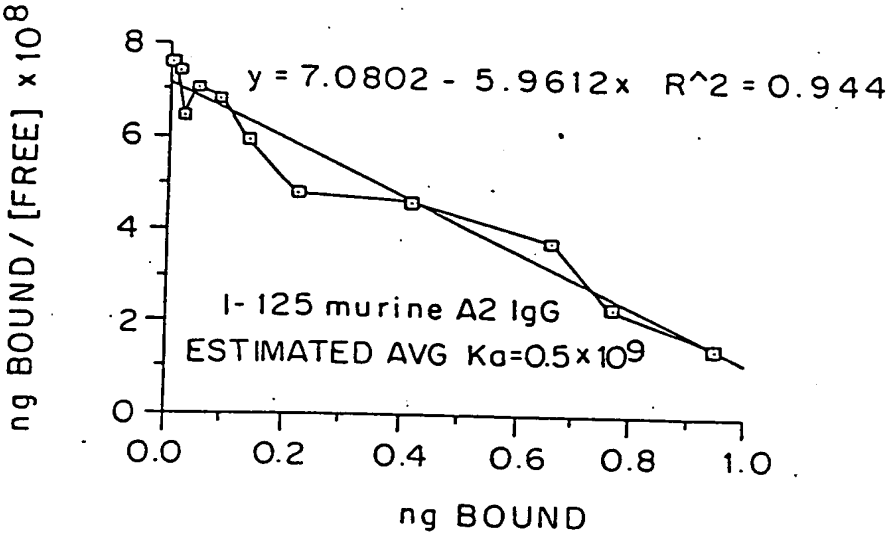


FIG. 10b

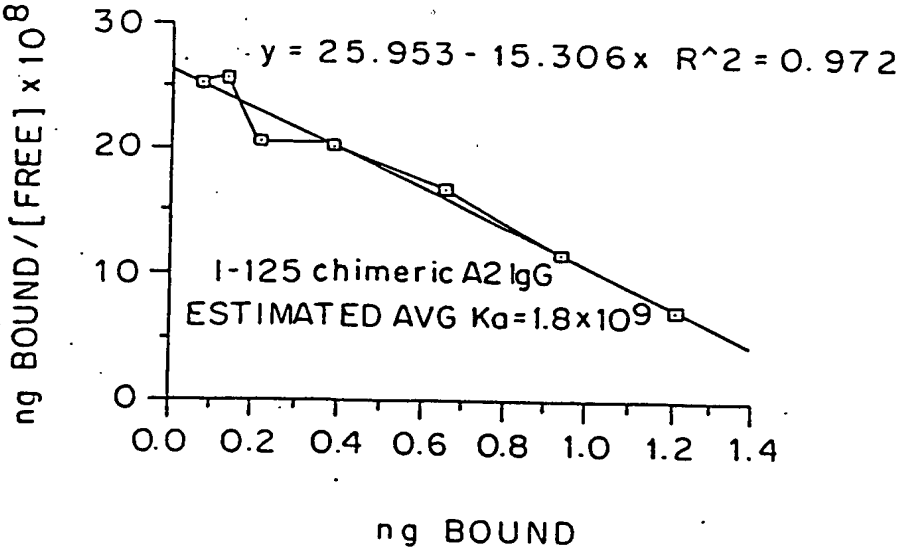


FIG. 11

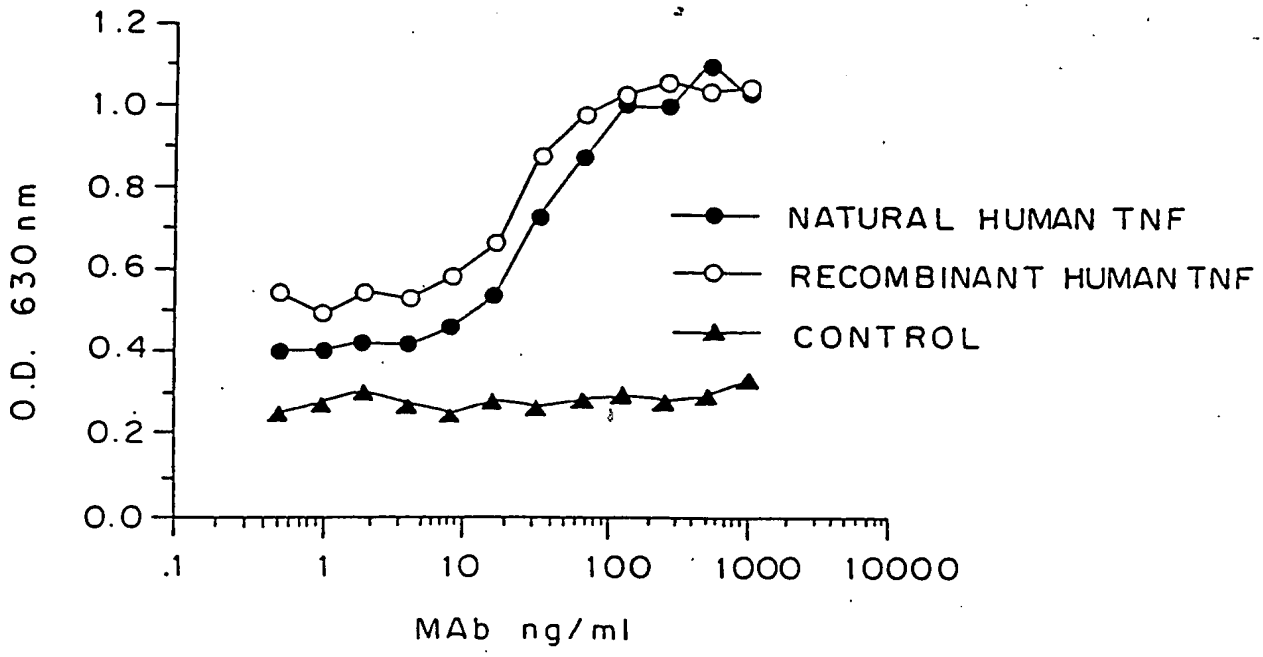

1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D. 630nm
.1
1
10
100
1000
10000
MAb ng/ml
NATURAL HUMAN TNF
RECOMBINANT HUMAN TNF
CONTROL

FIG. 12

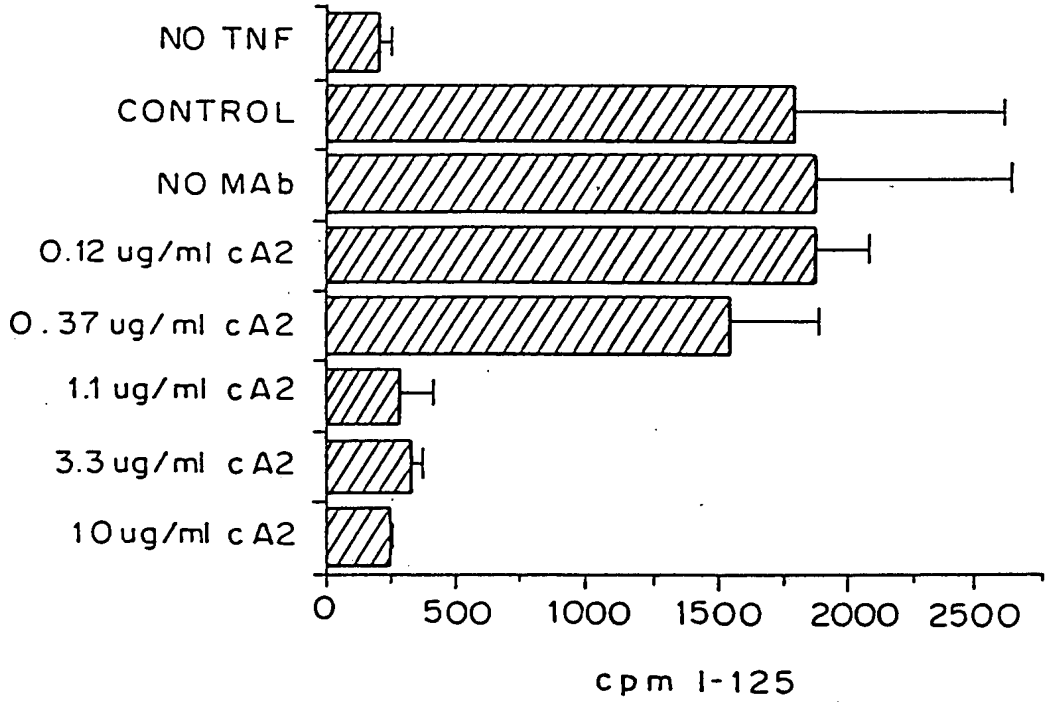

NO TNF
CONTROL
NO MAb
0.12 ug/ml cA2
0.37 ug/ml cA2
1.1 ug/ml cA2
3.3 ug/ml cA2
10 ug/ml cA2
0
500
1000
1500
2000
2500
cpm I-125

FIG. 13

```
1                                                   10
Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro

21                                                  30
Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly

41                                                  50
Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser

61                                                  70
Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile

81                                                  90
Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro

101                                                 110
Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu

121                                                 130
Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp

141                                                 150
Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
```

08 192102

FIG. 14a

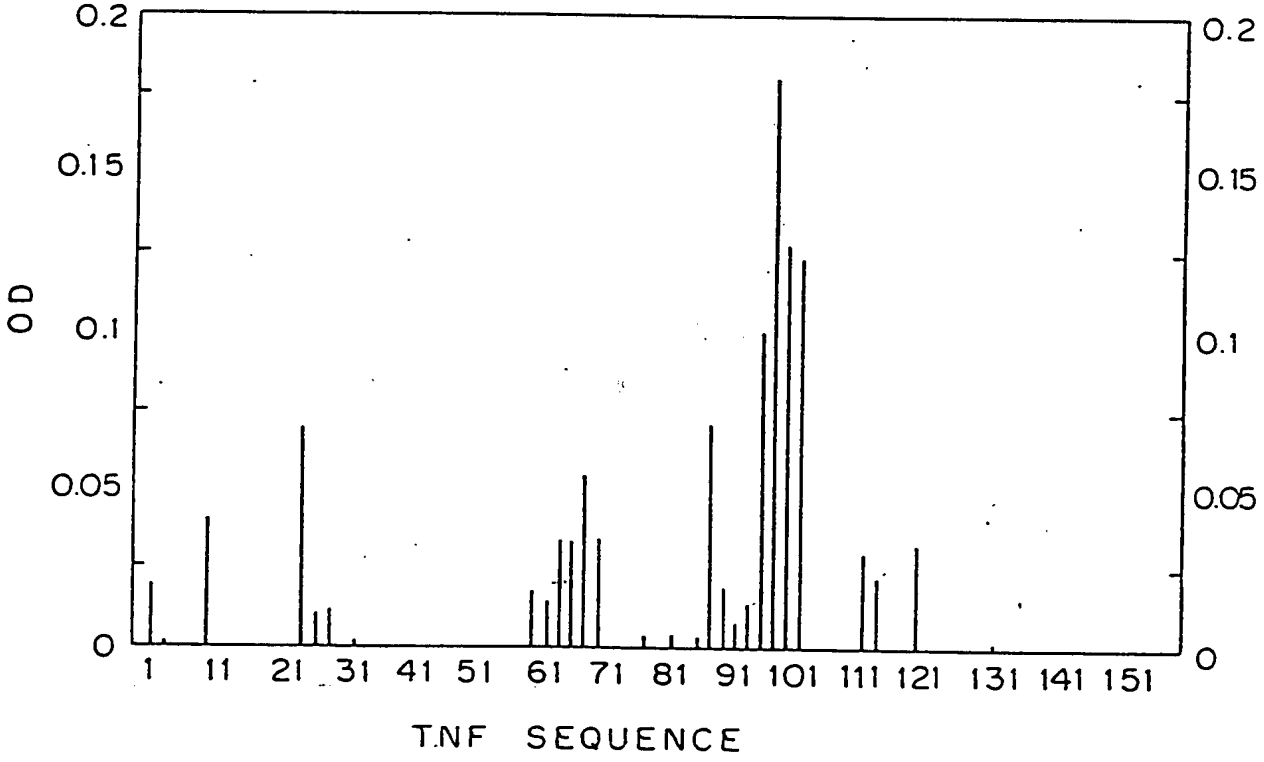


FIG. 14b

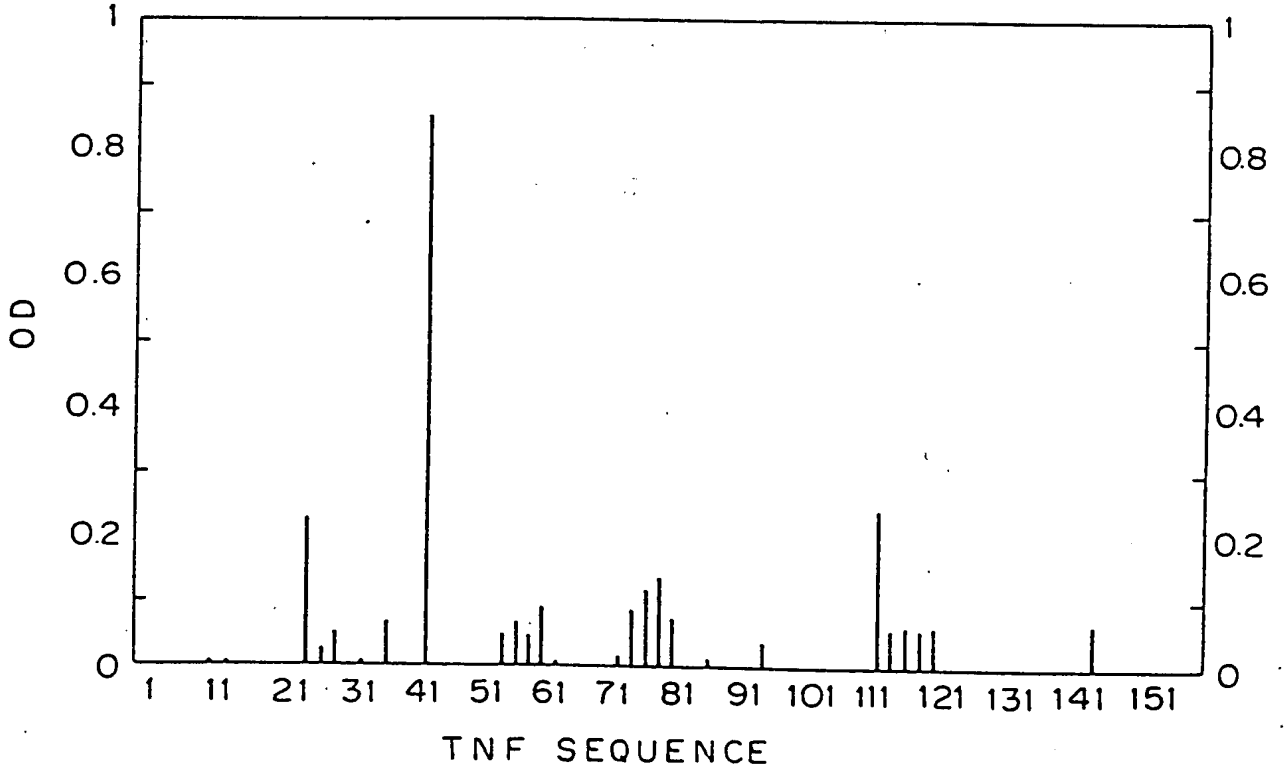
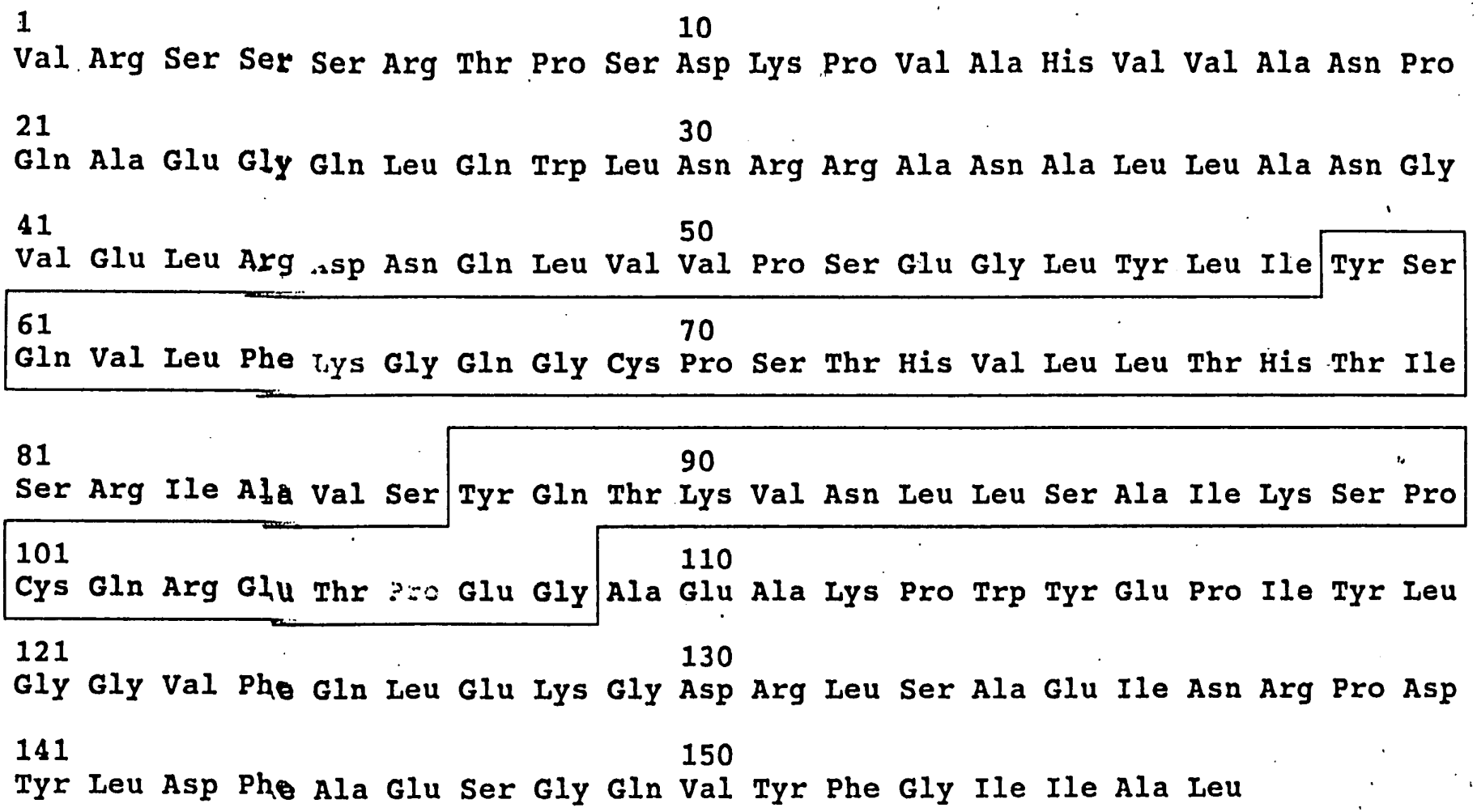


FIG. 15



08 192102

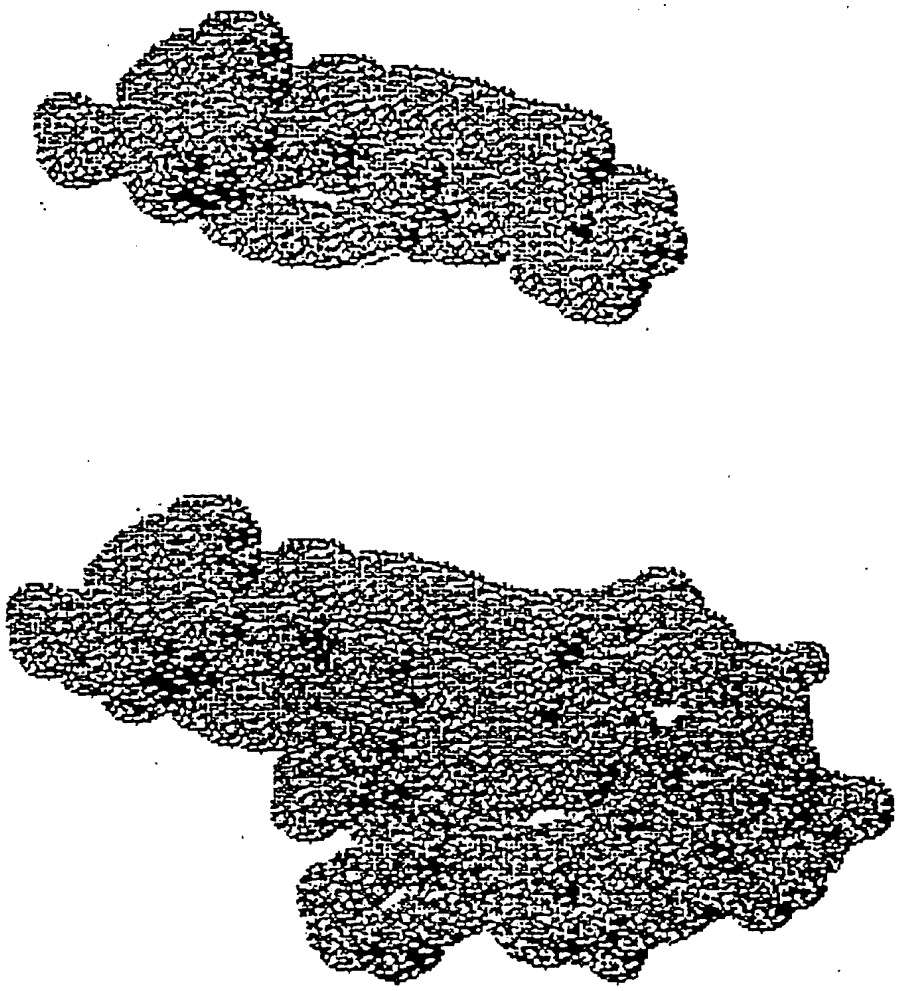


Figure 16

Figure 17A

CA2 LIGHT CHAIN VARIABLE REGION SEQUENCE

GACATCTTGCTCACTCAGTCTCCAGCCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGT
AspIleLeuLeuThrGlnSerProAlaIleLeuSerValSerProGlyGluArgValSer

TTCTCCTGCAGGGCCAGTCAGTTCGTTGGCTCAAGCATCCACTGGTATCAGCAAAGAACA
PheSerCysArgAlaSerGlnPheValGlySerSerIleHisTrpTyrGlnGlnArgThr

AATCGTTCTCCAAGGCTTCTCATAAAGTATGCTTCTGAGTCTATGTCTGGGATCCCTTCC
AsnGlySerProArgLeuLeuIleLysTyrAlaSerGluSerMetSerGlyIleProSer

AGGTTTAGTGGCAGTGGATCAGGGACAGATTTTACTCTTAGCATCAACACTGTGGAGTCT
ArgPheSerGlySerGlySerGlyThrAspPheThrLeuSerIleAsnThrValGluSer

GAAGATATTGCAGATTATTACTGTCAAGAAAGTCATAGCTGGCCCATTCACGTTCGGCTCG
GluAspIleAlaAspTyrTyrCysGlnGlnSerHisSerTrpProPheThrPheGlySer

GGGACAAATTTGGAAGTAAAA
GlyThrAsnLeuGluValLys

```
CAAGTCAAGCTTCACCACTCTCCACCAGCCCTTCCTCCAACCTCCACCATCCATCAAACTC
GluValLysLeuGluGluSerGlyGlyGlyLeuValGlnProGlyGlySerMetLysLeu

TCCTGTGTTGCCTCTGGATTCATTTTCAGTAACCACTGGATGAACTGGGTCCGCCAGTCT
SerCysValAlaSerGlyPheIlePheSerAsnHisTrpMetAsnTrpValArgGlnSer

CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGATCAAAATCTATTAATTCTGCAACA
ProGluLysGlyLeuGluTrpValAlaGluIleArgSerLysSerIleAsnSerAlaThr

CATTATGCGGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGAGATGATTCCAAAAGTGCT
HisTyrAlaGluSerValLysGlyArgPheThrIleSerArgAspAspSerLysSerAla

CTCTACCTGCAAATGACCCACTTAAGAACTGAAGACACTGGCGTTTATTACTGTTCCAGG
ValTyrLeuGlnMetThrAspLeuArgThrGluAspThrGlyValTyrTyrCysSerArg

AATTACTACGGTAGTACCTACGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCC
AsnTyrTyrGlySerThrTyrAspTyrTrpGlyGlnGlyThrThrLeuThrValSer
```

Figure 17B

anti-TNF Trial

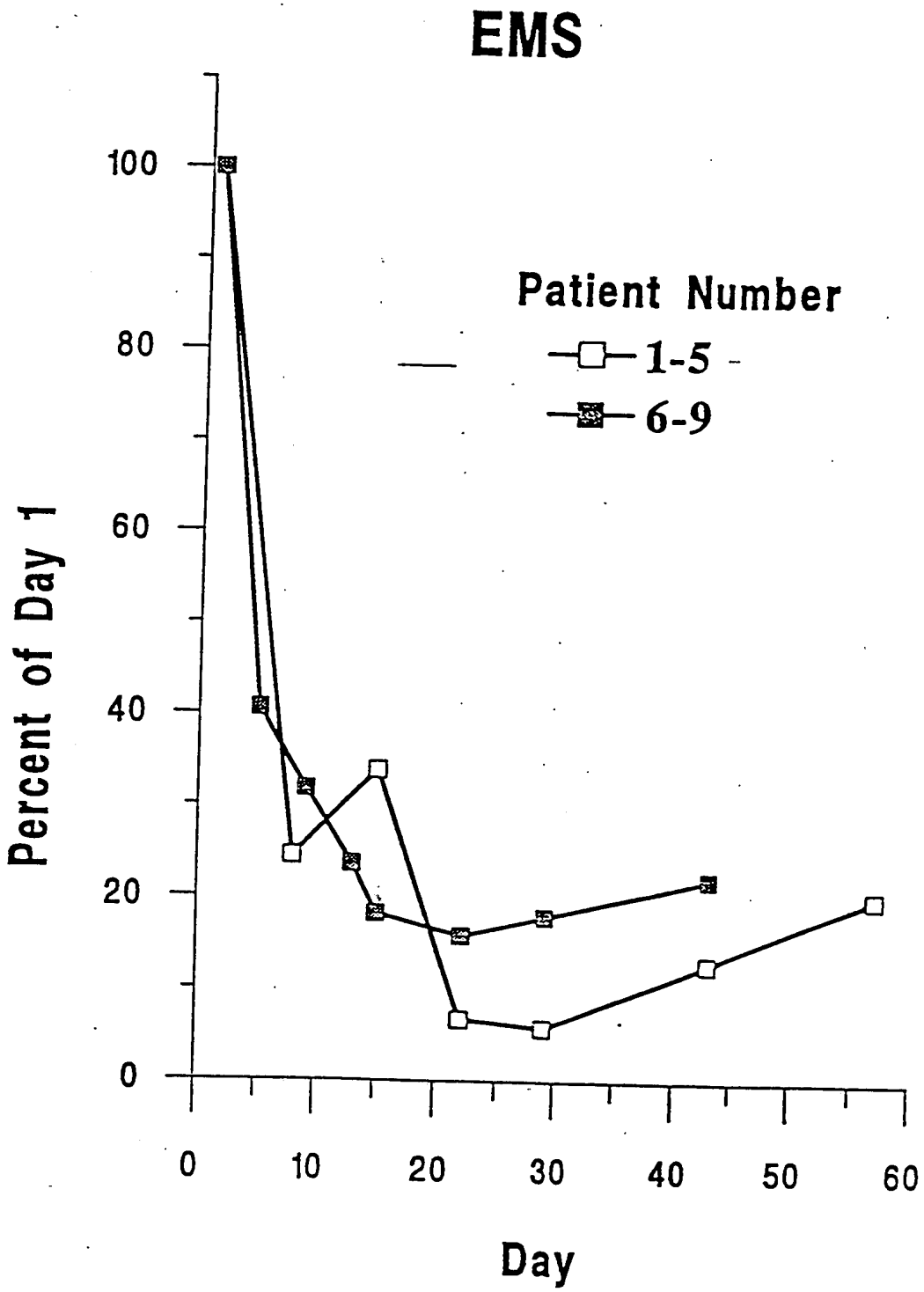


FIG-18

anti-TNF Trial

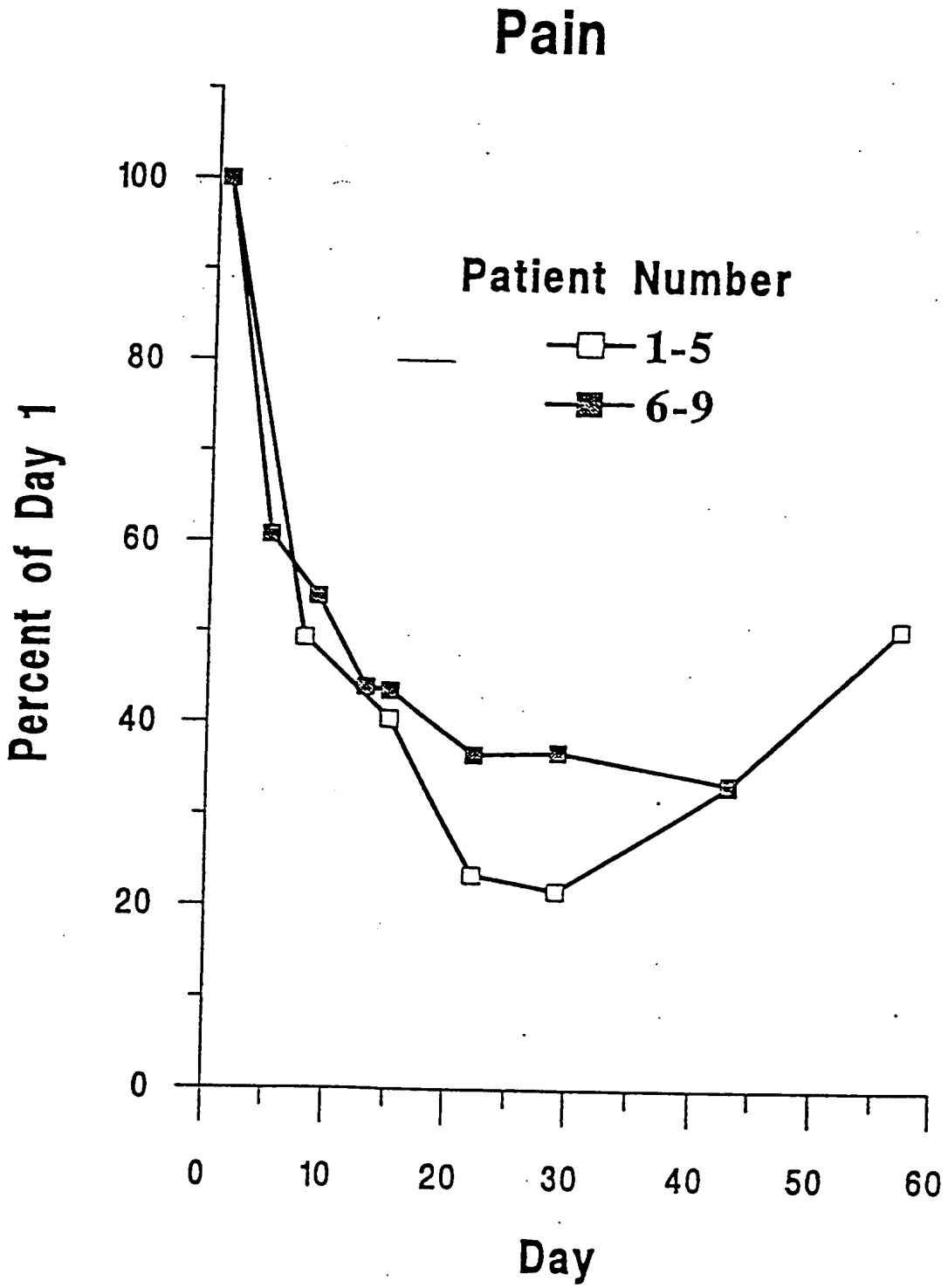


FIG. 19

anti-TNF Trial

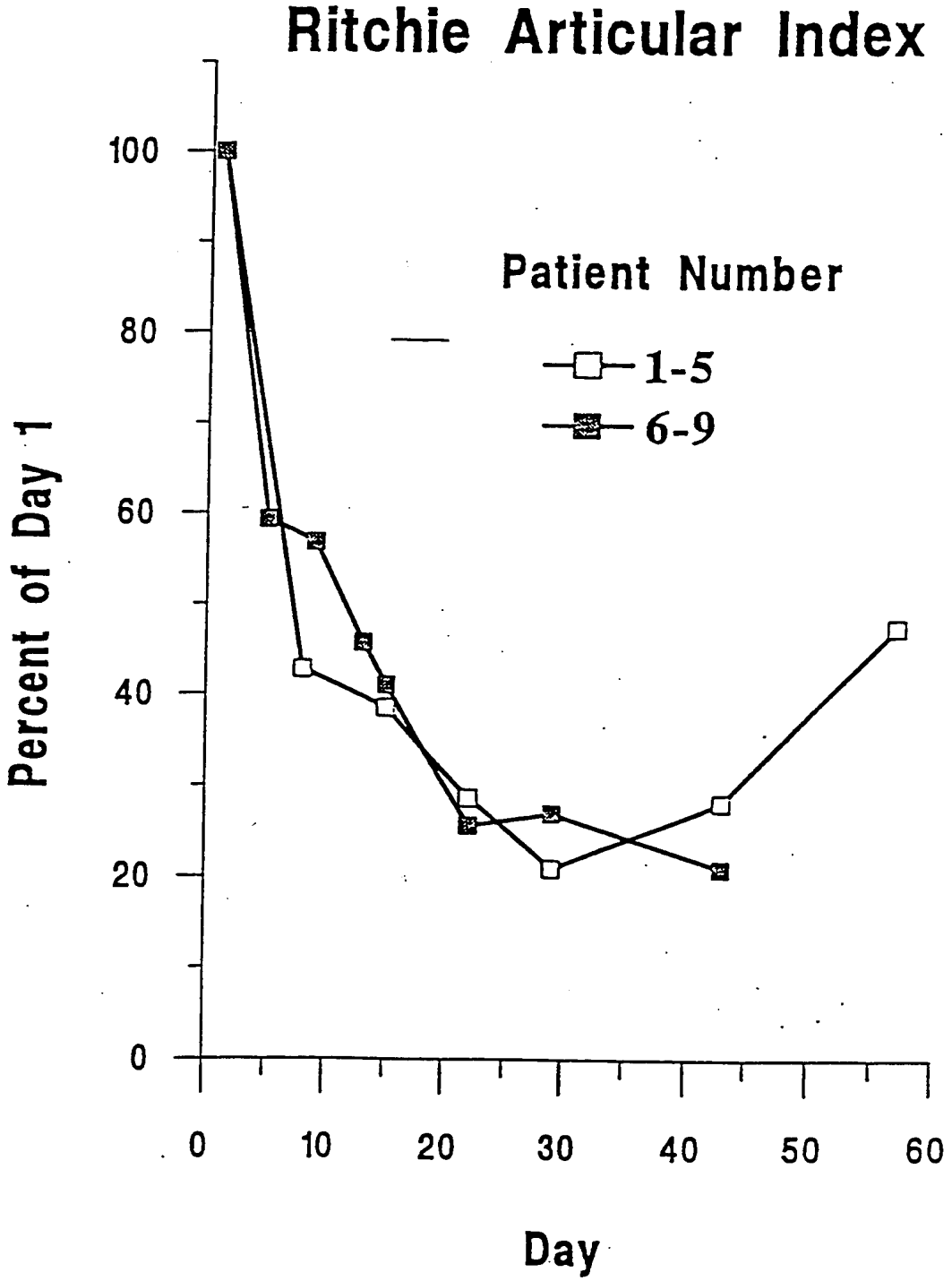


FIG. 20

anti-TNF Trial

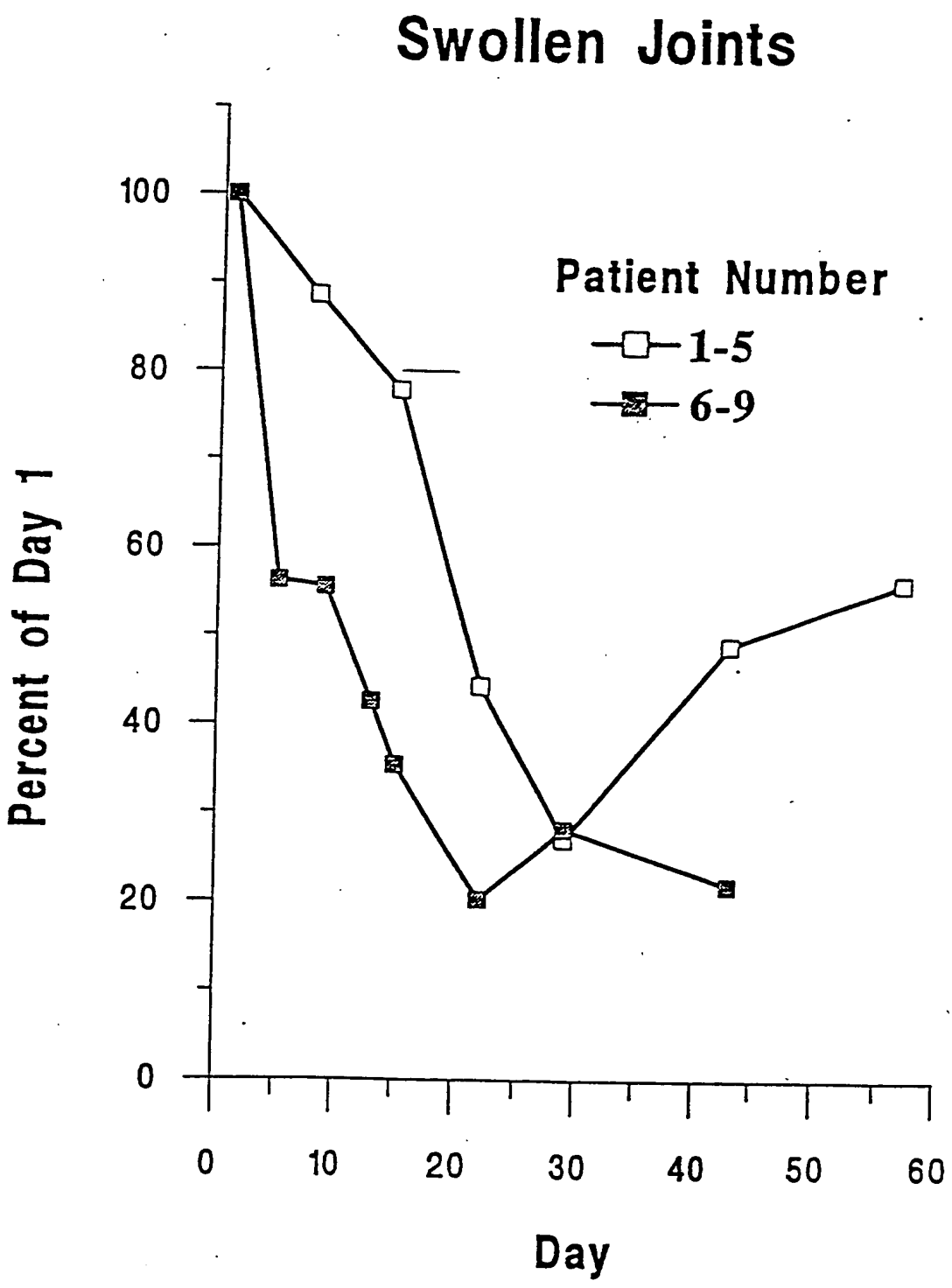


FIG 21

anti-TNF Trial

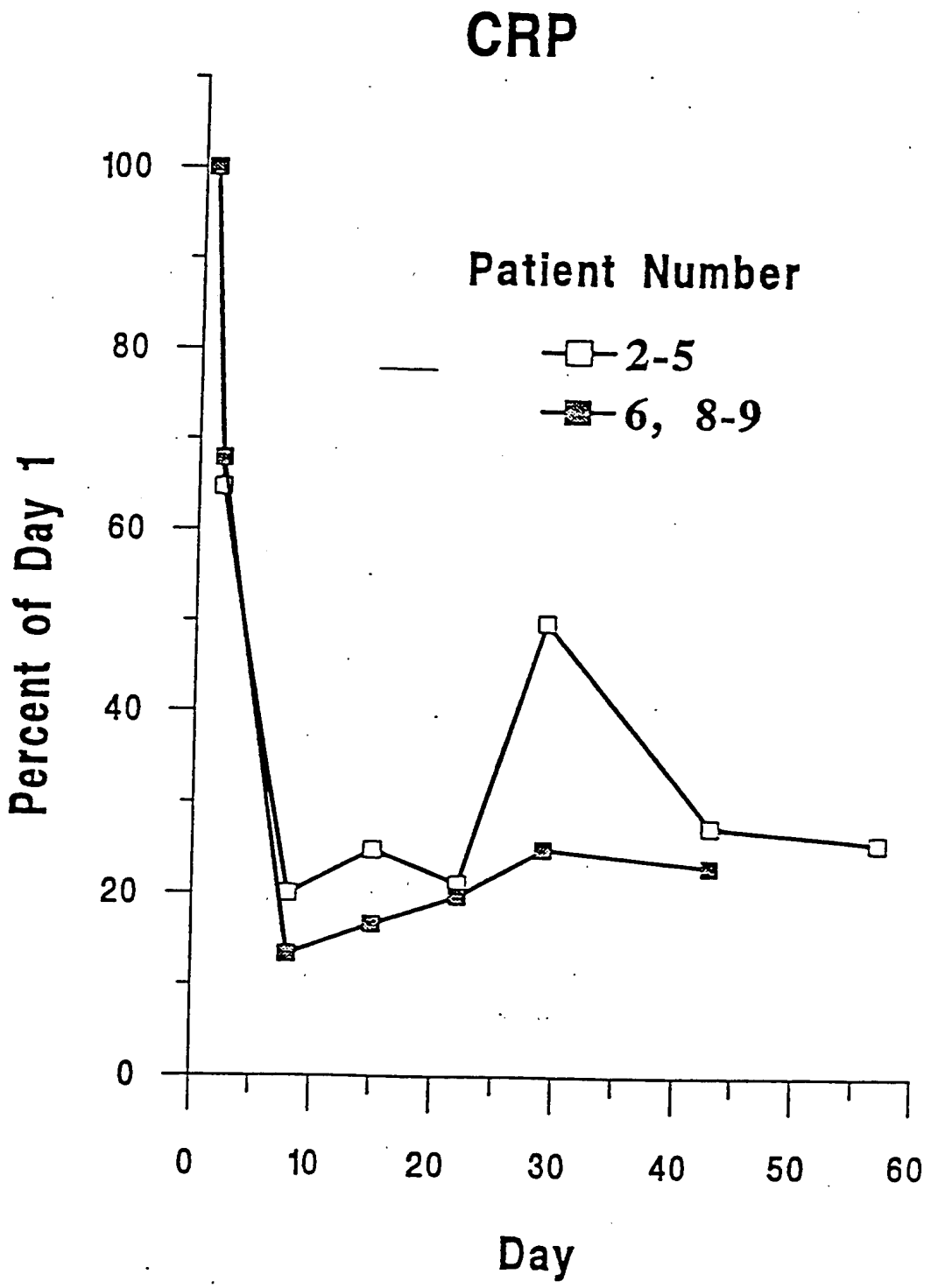


FIG. 22

anti-TNF Trial

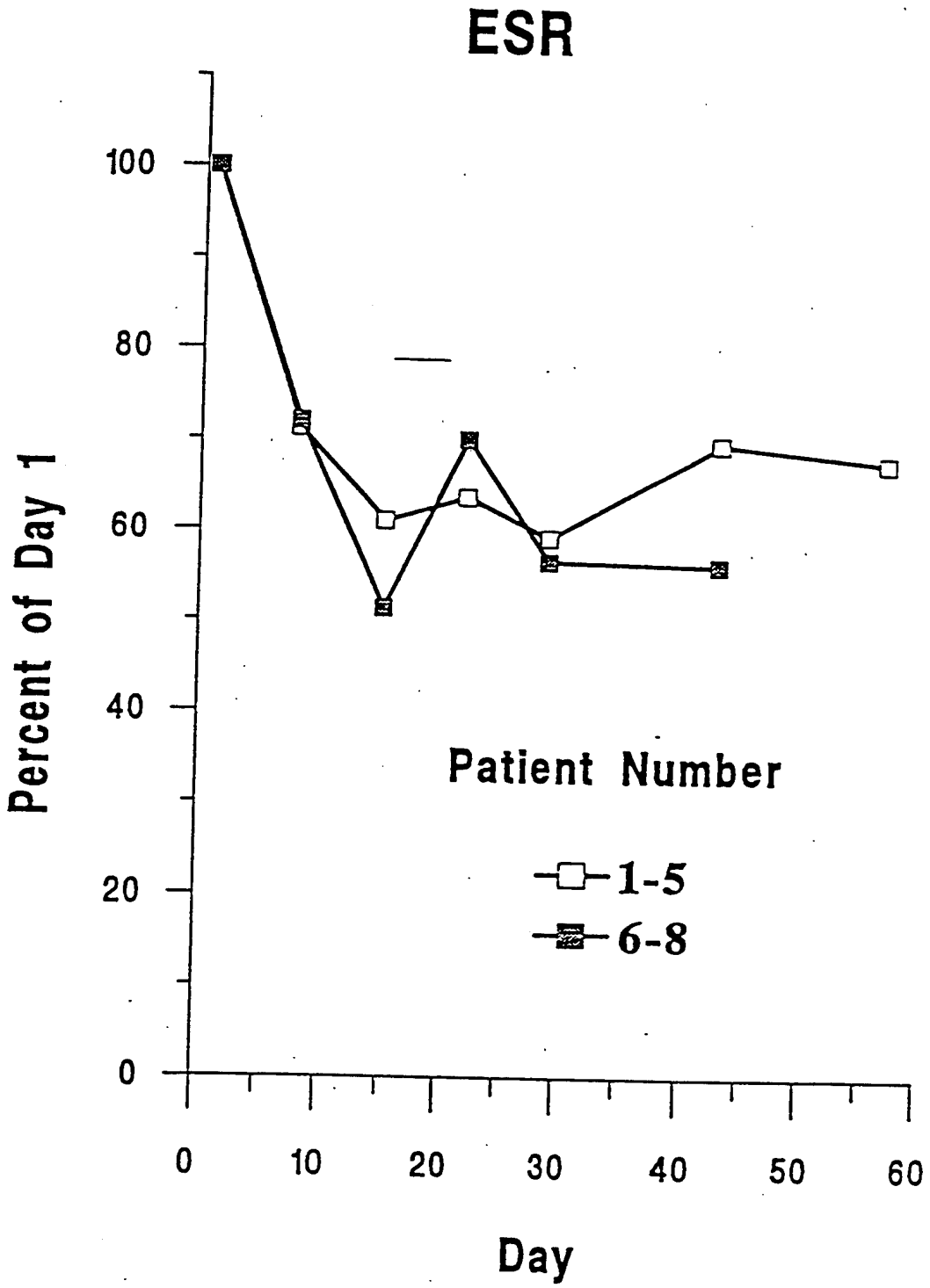


FIG 23

anti-TNF Trial

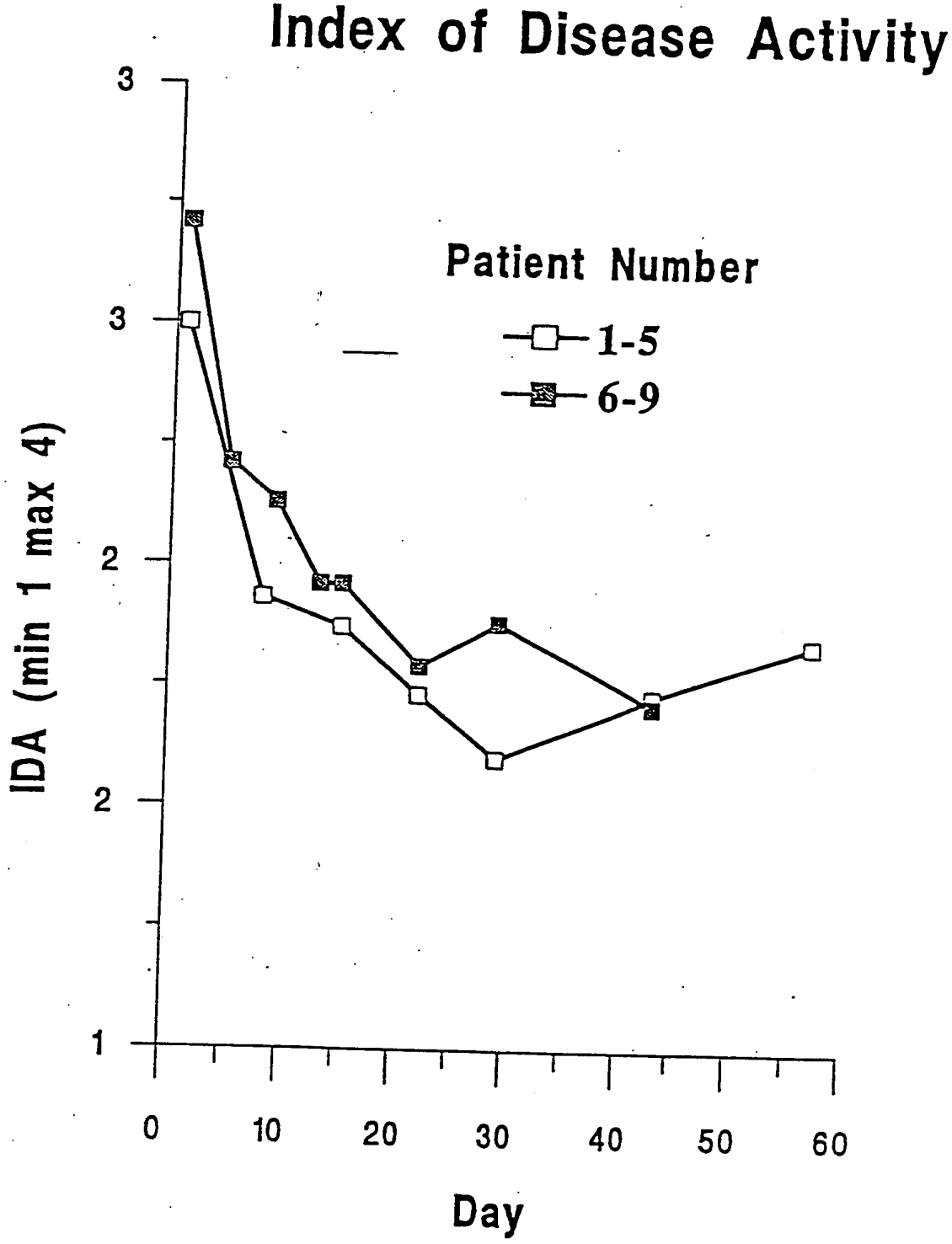


FIG 24

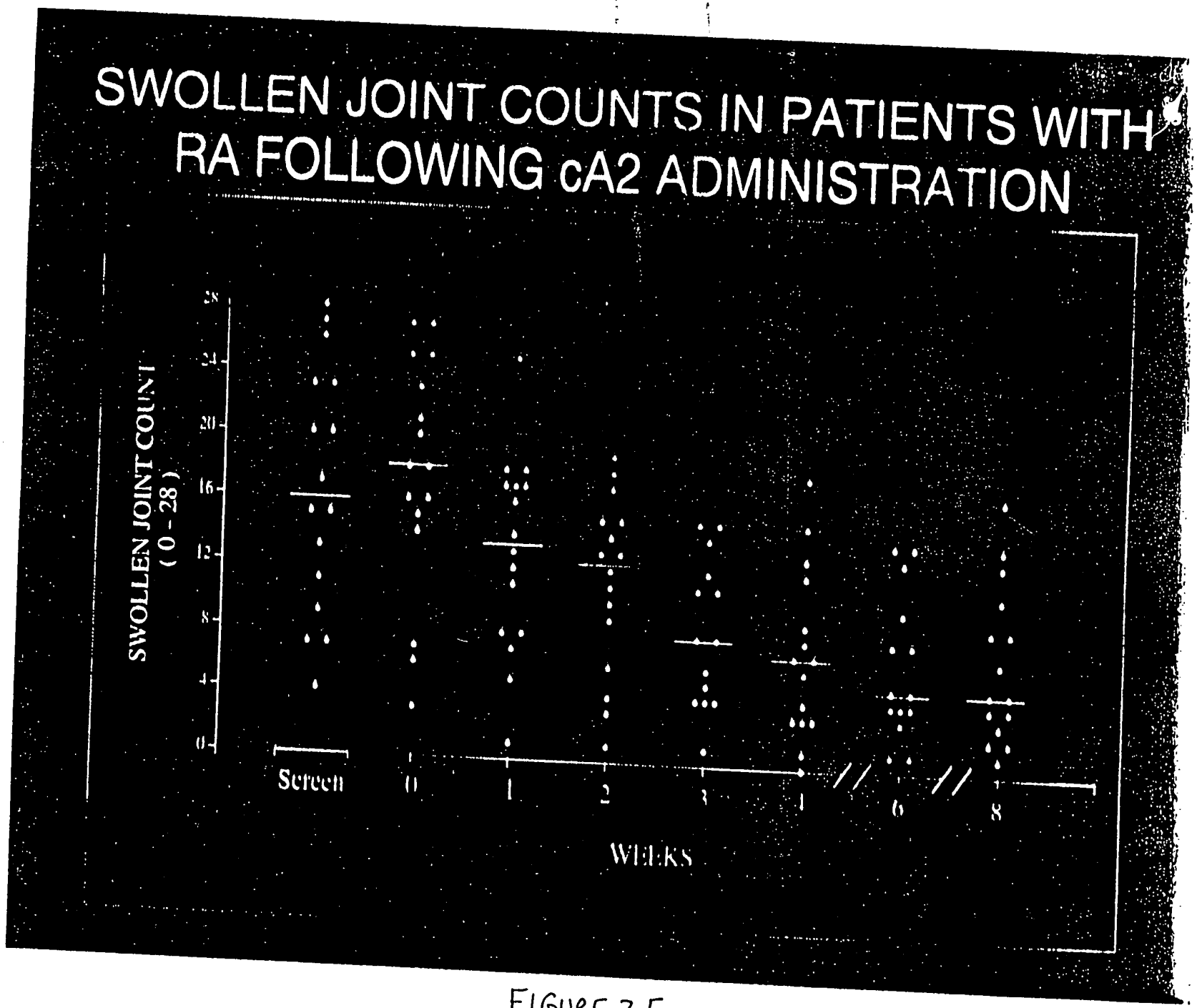


FIGURE 25

20102 80 193102

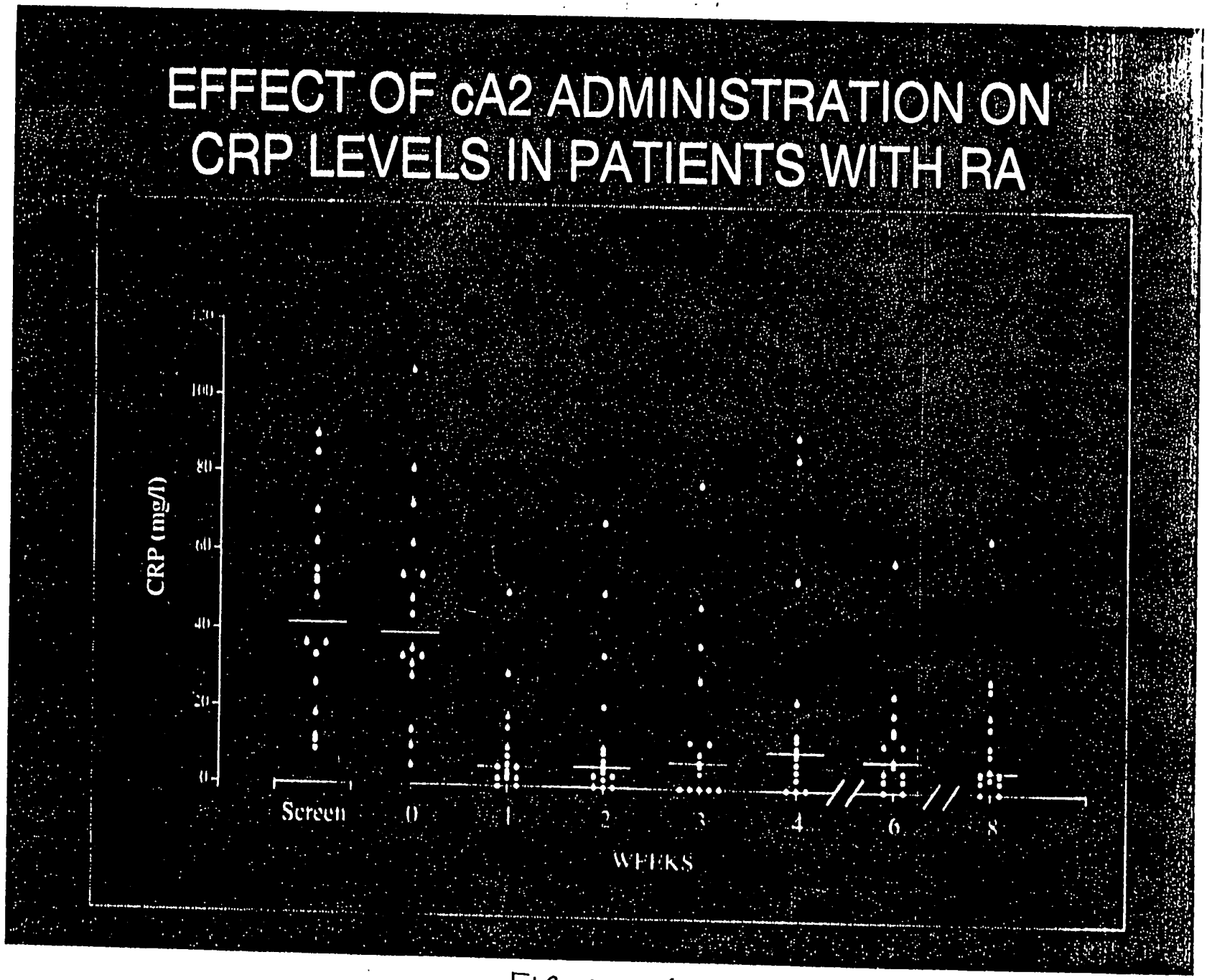
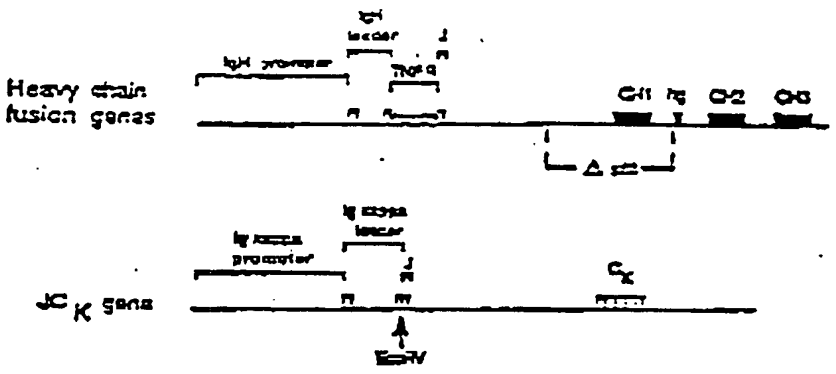


FIGURE 26

US 192102

27 A



27 B

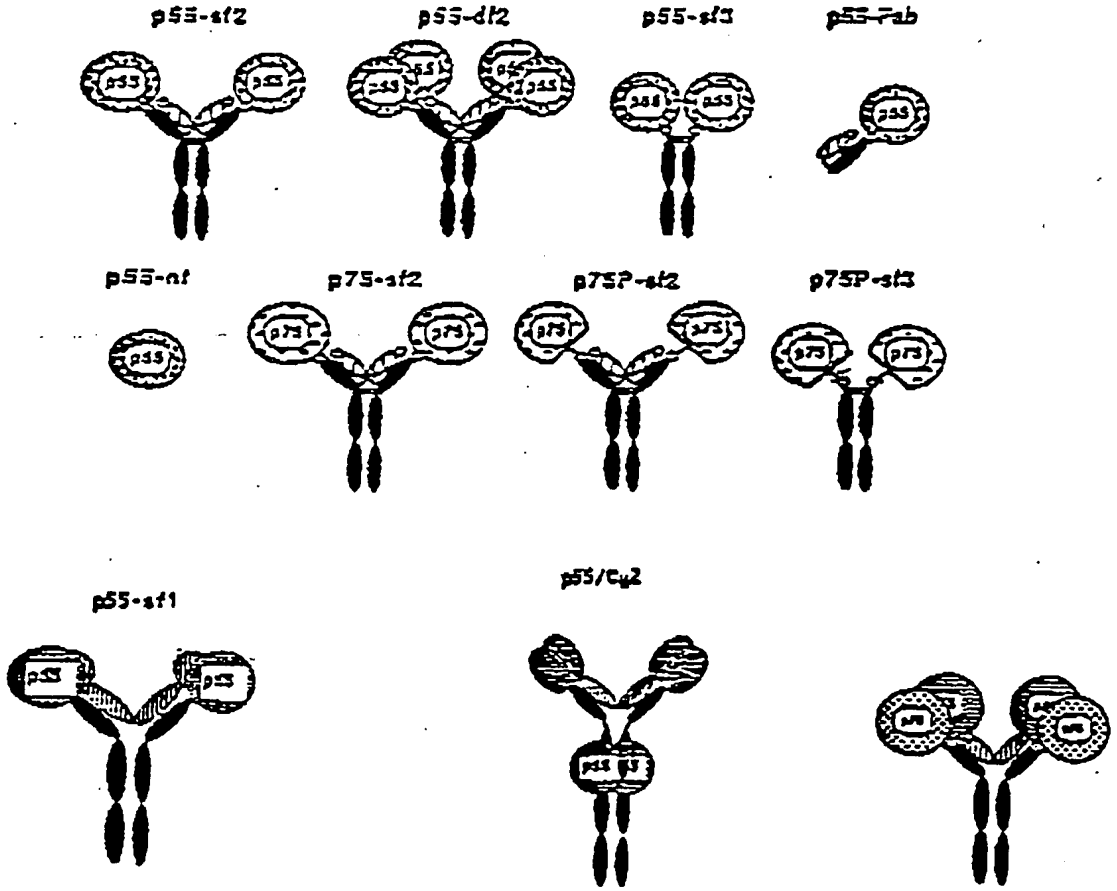


FIGURE 27 A-B

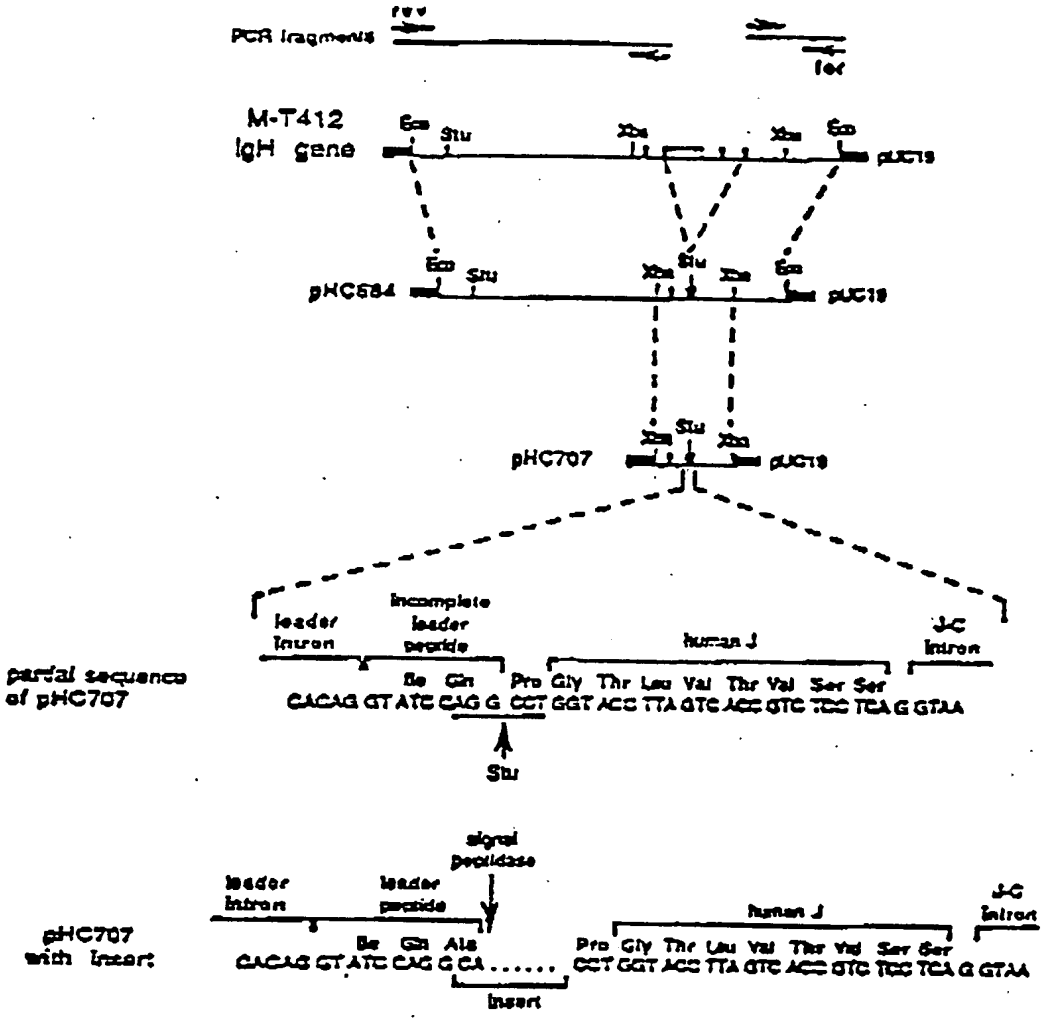

PCR fragments
M-T412
IgH gene
pHC584
pHC707
leader
Intron
Incomplete
leader
peptide
human J
J-C
Intron
partial sequence
of pHC707
Ile Gln Pro Gly Thr Leu Val Thr Val Ser Ser
CACAG GT ATC CAG G CCT GGT ACC TTA GTC ACC GTC TCC TCA G GTAA
Stu
signal
peptidase
leader
Intron
leader
peptide
human J
J-C
Intron
pHC707
with Insert
Ile Gln Ala
CACAG GT ATC CAG G CA .....
Pro Gly Thr Leu Val Thr Val Ser Ser
CCT GGT ACC TTA GTC ACC GTC TCC TCA G GTAA
Insert

FIGURE 28

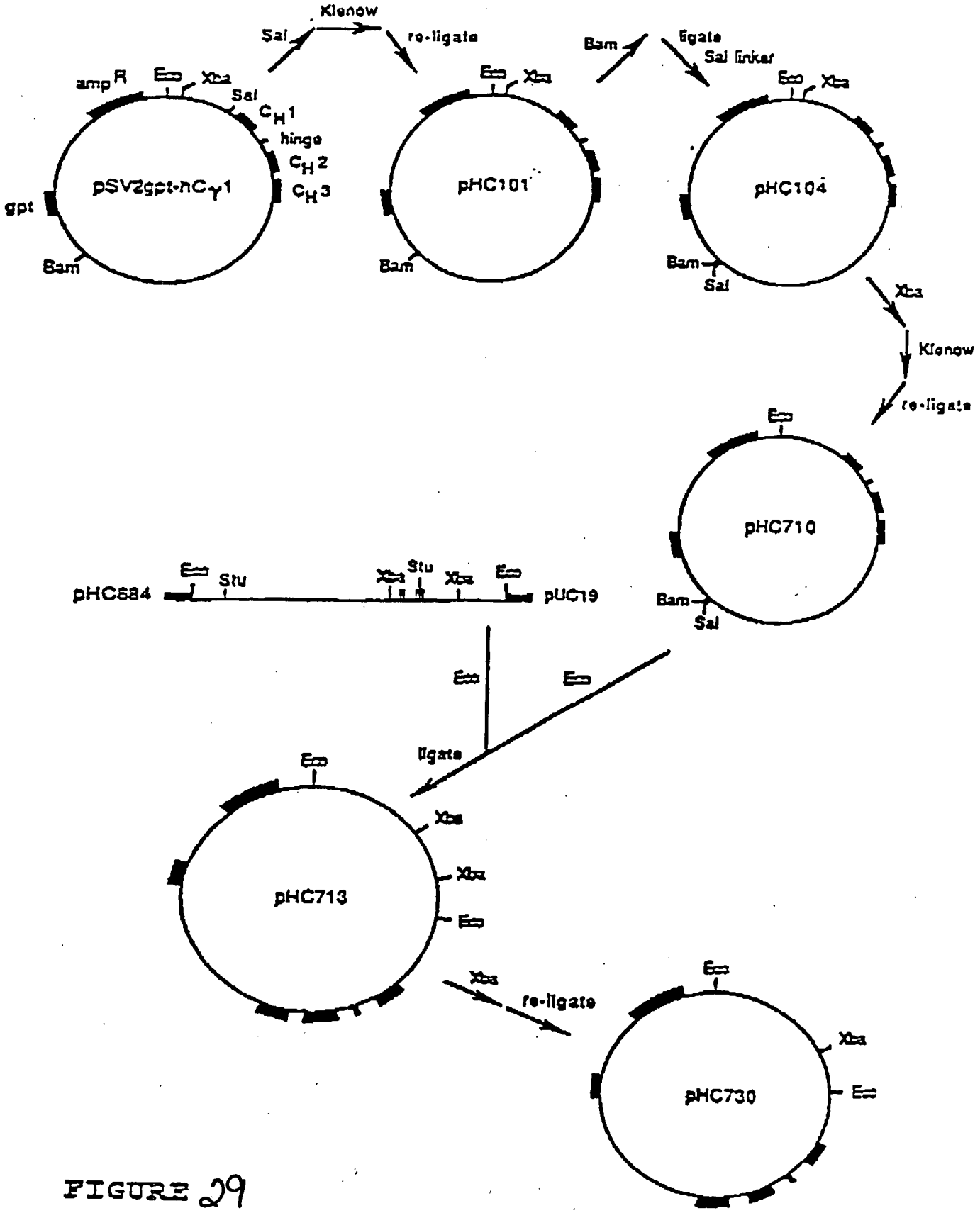

Klenow
Sal
re-ligate
amp R
Eco
Xba
Sal
C_H1
hinge
C_H2
C_H3
pSV2gpt-hCγ1
gpt
Bam
pHC101
Bam
ligate
Sal linker
pHC104
Bam
Sal
Xba
Klenow
re-ligate
pHC710
Bam
Sal
pHC684
Eco
Stu
Xba
Stu
Xba
Eco
pUC19
Eco
Eco
ligate
pHC713
Xba
Xba
Eco
Xba
re-ligate
pHC730
Xba
Eco

FIGURE 29

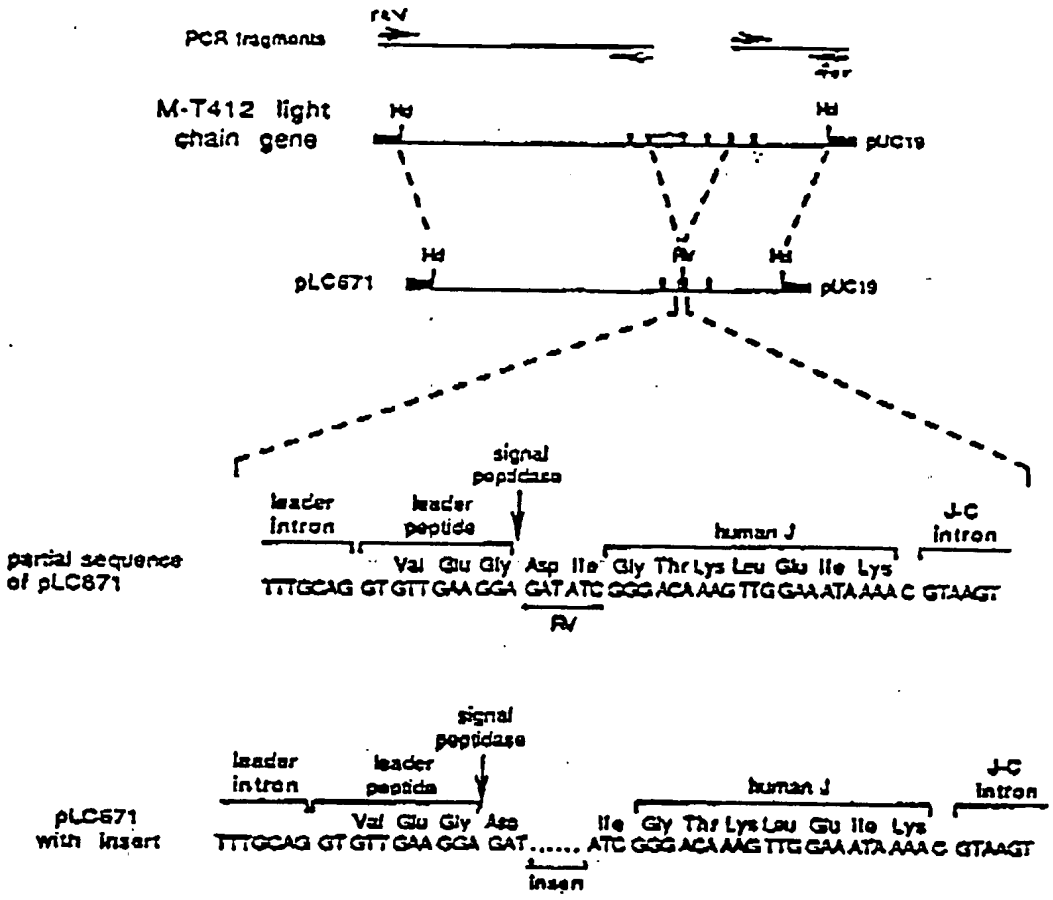

PCR fragments
M-T412 light chain gene
Hd
pUC19
pLC671
Pv
J L
signal peptidase
leader intron
leader peptide
human J
J-C intron
partial sequence of pLC671
Val Glu Gly Asp Ile Gly Thr Lys Leu Glu Ile Lys
TTTGCAG GT GTT GAA GGA GAT ATC GGG ACA AAG TTG GAA ATA AAA C GTAAGT
pLC671 with insert
insert

FIGURE 30

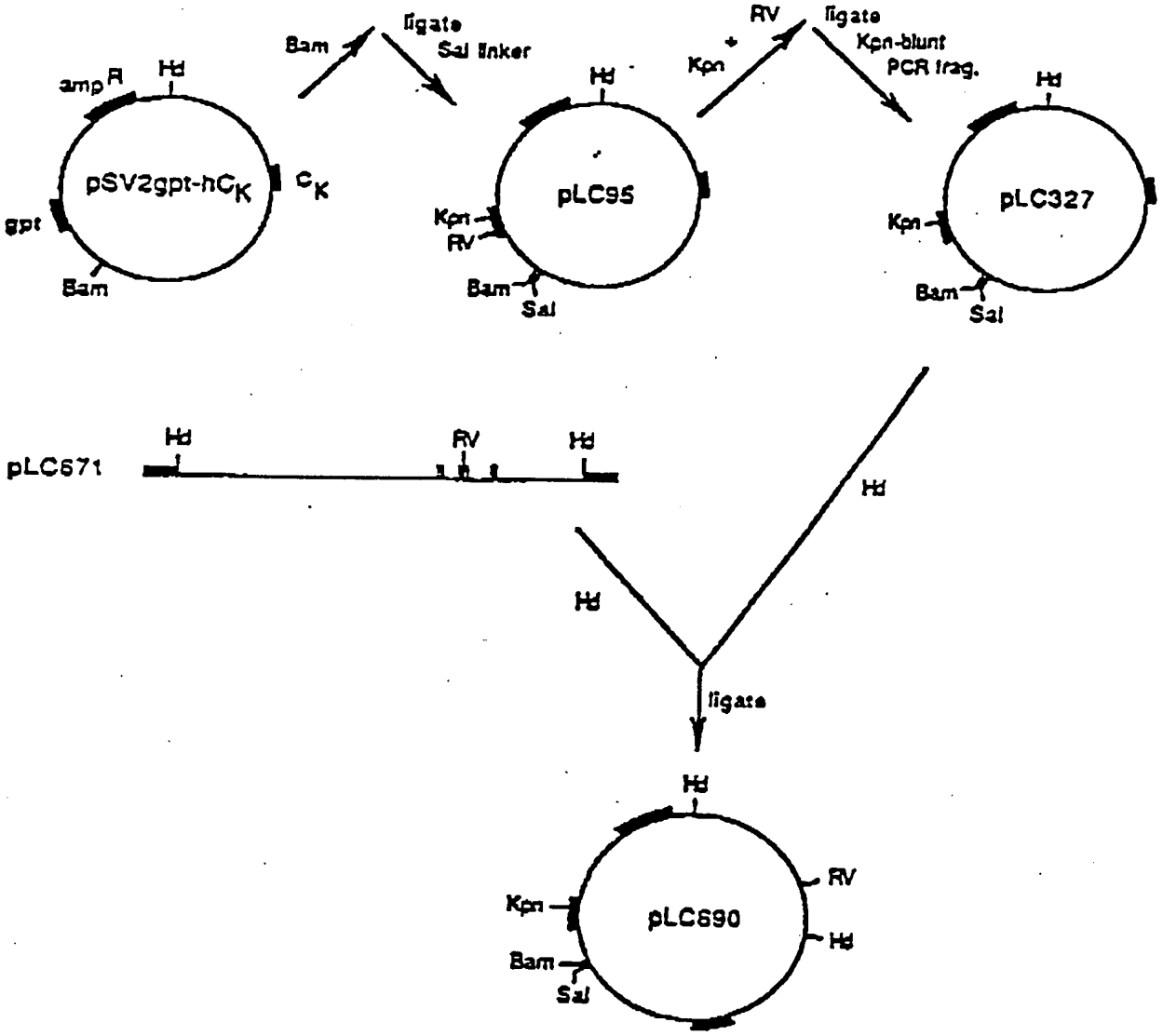

amp R
Hd
pSV2gpt-hC$_K$
C$_K$
gpt
Bam
ligate
Sal linker
pLC95
Kpn
RV
Sal
RV
+
Kpn
ligate
Kpn-blunt
PCR frag.
pLC327
pLC671
Hd
RV
Hd
Hd
Hd
ligate
pLC690
Kpn
Bam
Sal
RV
Hd

FIGURE 31

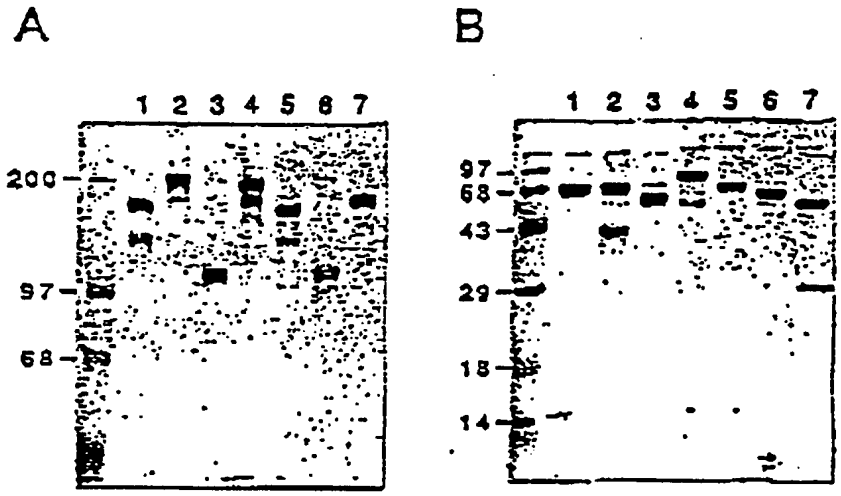


FIGURE 32

A)

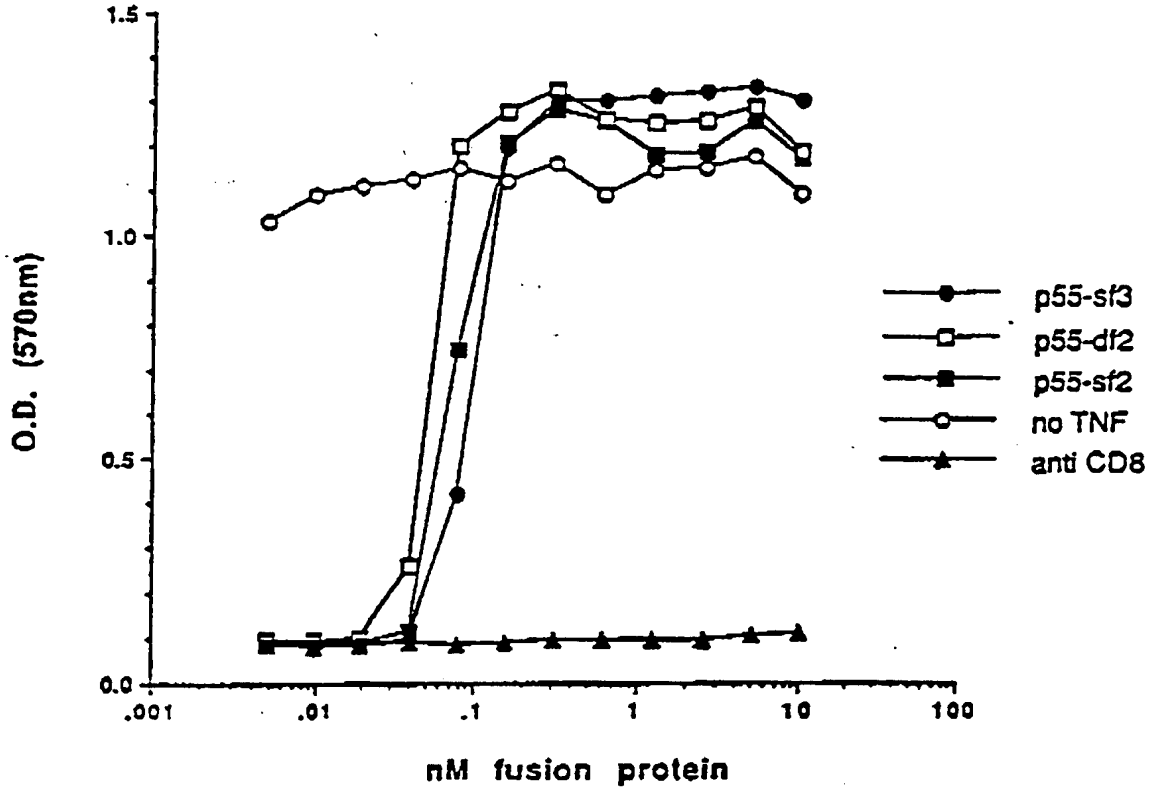

1.5
1.0
0.5
0.0
O.D. (570nm)
.001
.01
.1
1
10
100
nM fusion protein
p55-sf3
p55-df2
p55-sf2
no TNF
anti CD8

FIGURE 33 A

B)

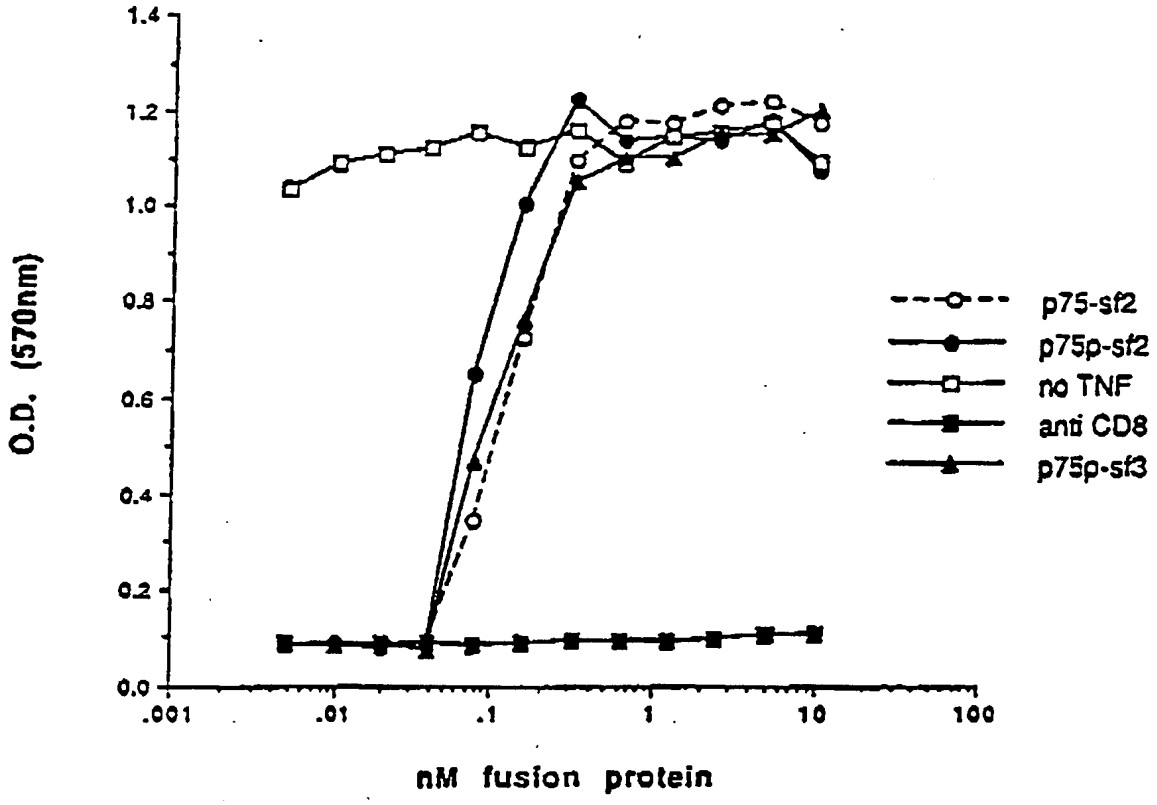

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D. (570nm)
.001
.01
.1
1
10
100
nM fusion protein
p75-sf2
p75p-sf2
no TNF
anti CD8
p75p-sf3

FIGURE 33 B

C)

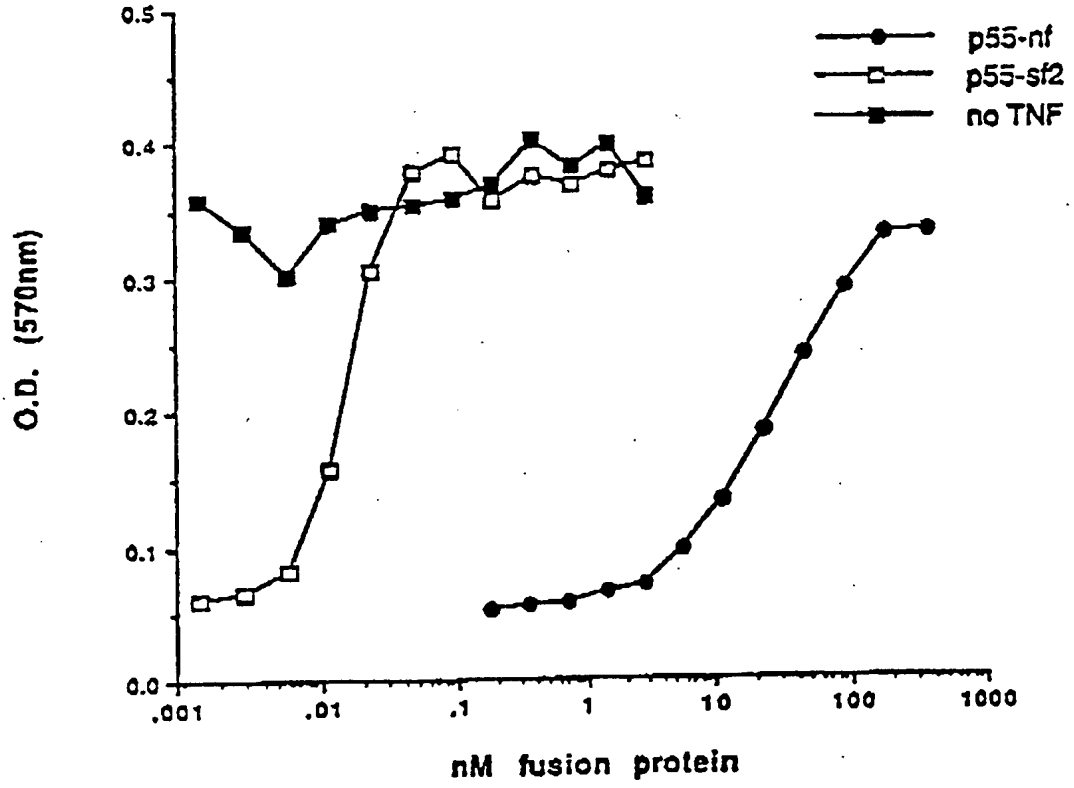

p55-nf
p55-sf2
no TNF
O.D. (570nm)
0.5
0.4
0.3
0.2
0.1
0.0
.001
.01
.1
1
10
100
1000
nM fusion protein

FIGURE 33 C

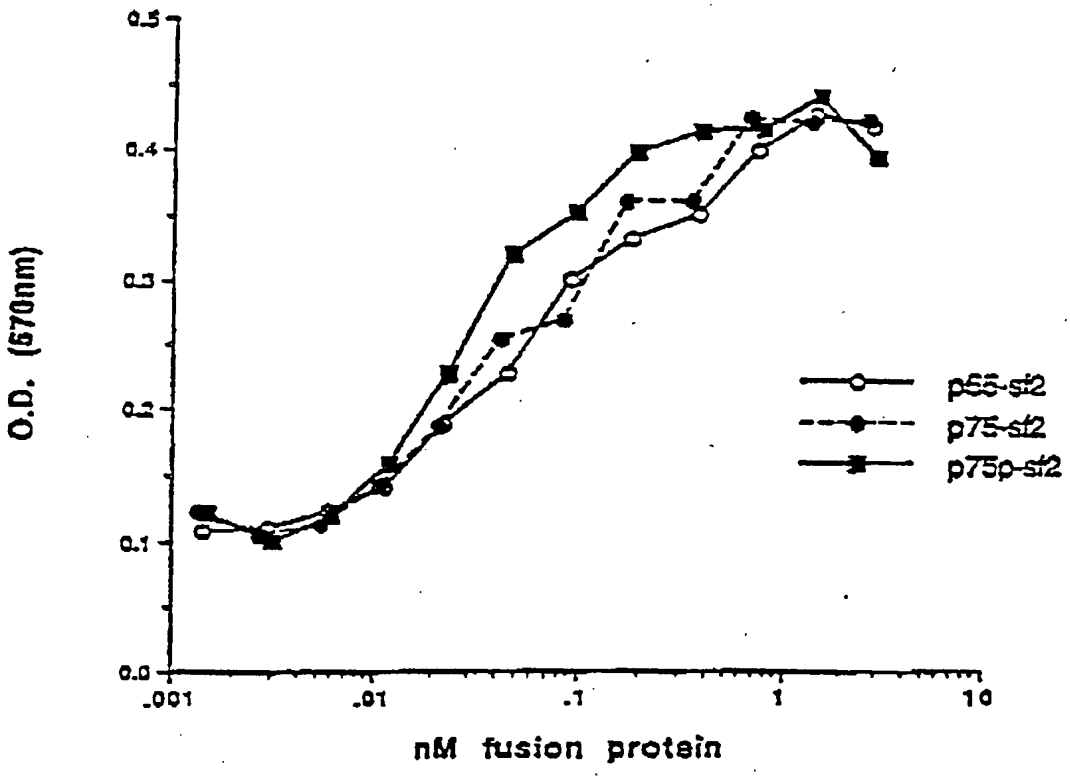
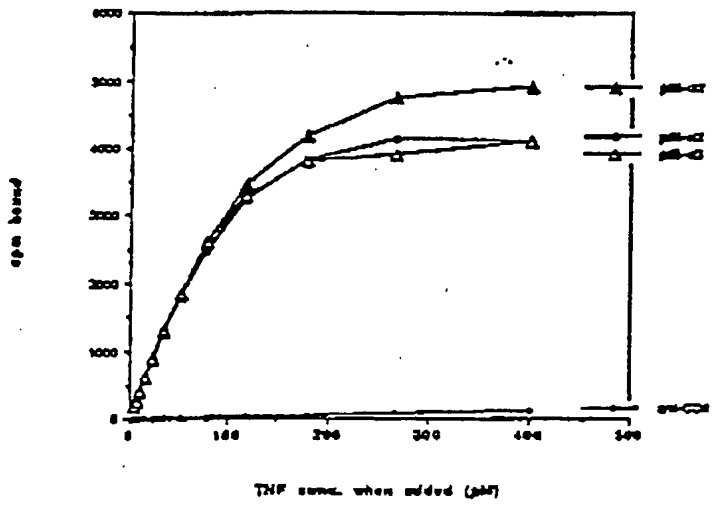

0.5
0.4
0.3
0.2
0.1
0.0
O.D. (570nm)
.001
.01
.1
1
10
nM fusion protein
p55-sf2
p75-sf2
p75p-sf2

FIGURE 34

A)



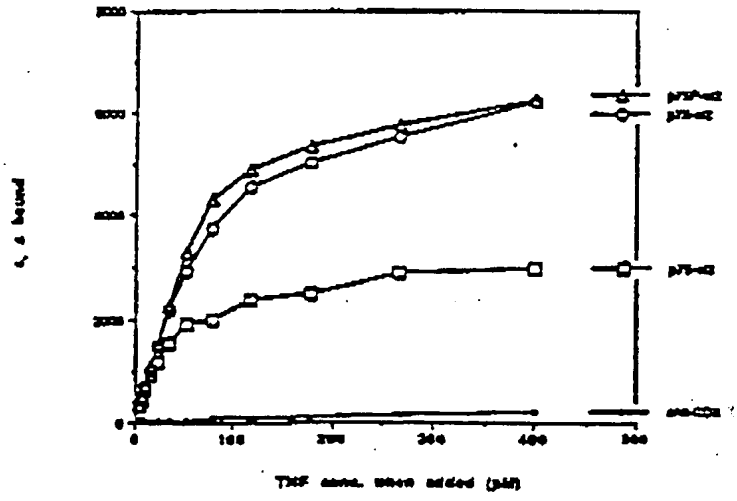


FIGURE 35 A

B)

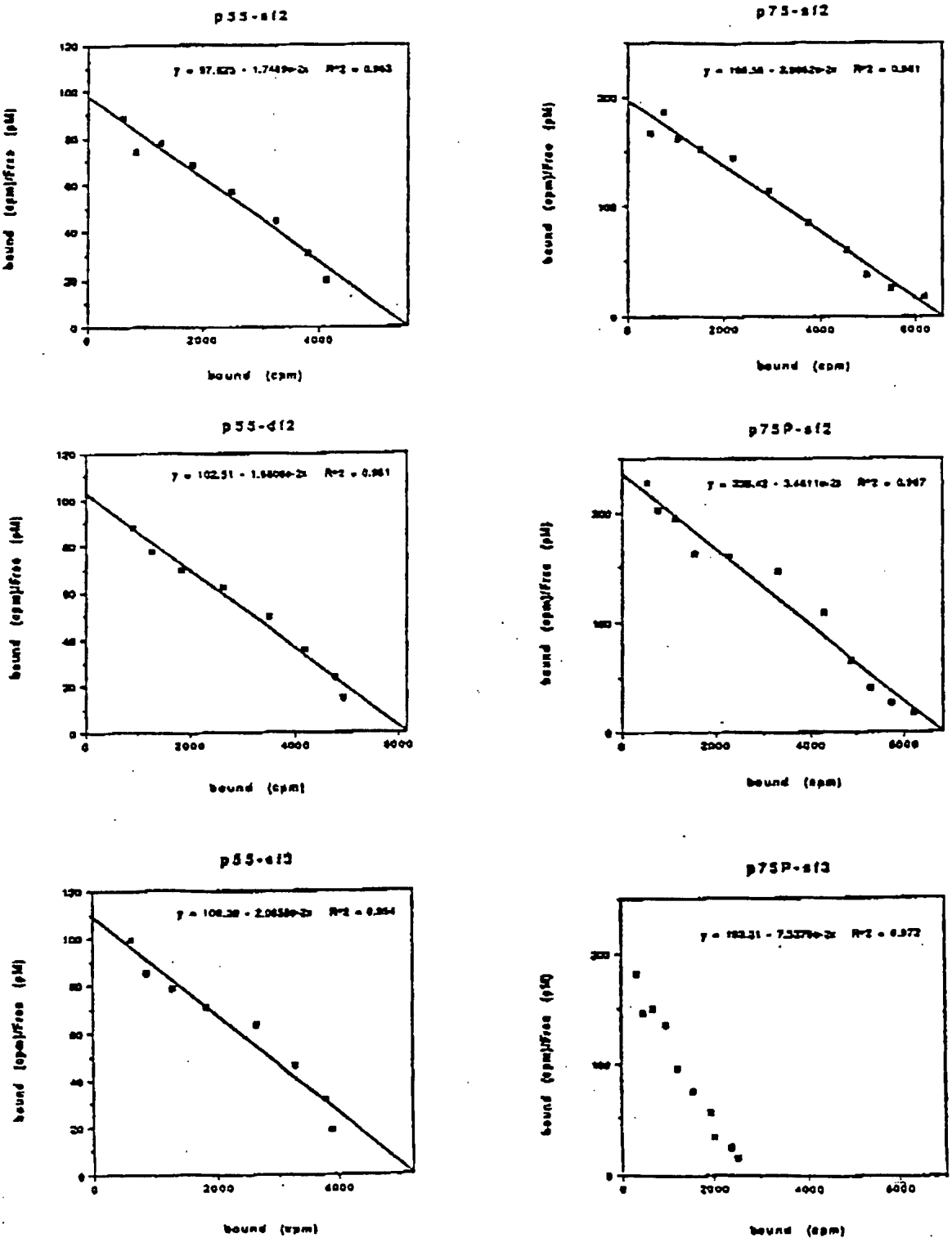

p55-sf2
bound (cpm)/free (pM)
bound (cpm)
p75-sf2
bound (cpm)/free (pM)
bound (cpm)
p55-df2
bound (cpm)/free (pM)
bound (cpm)
p75P-sf2
bound (cpm)/free (pM)
bound (cpm)
p55-sf3
bound (cpm)/free (pM)
bound (cpm)
p75P-sf3
bound (cpm)/free (pM)
bound (cpm)

FIGURE 35 B

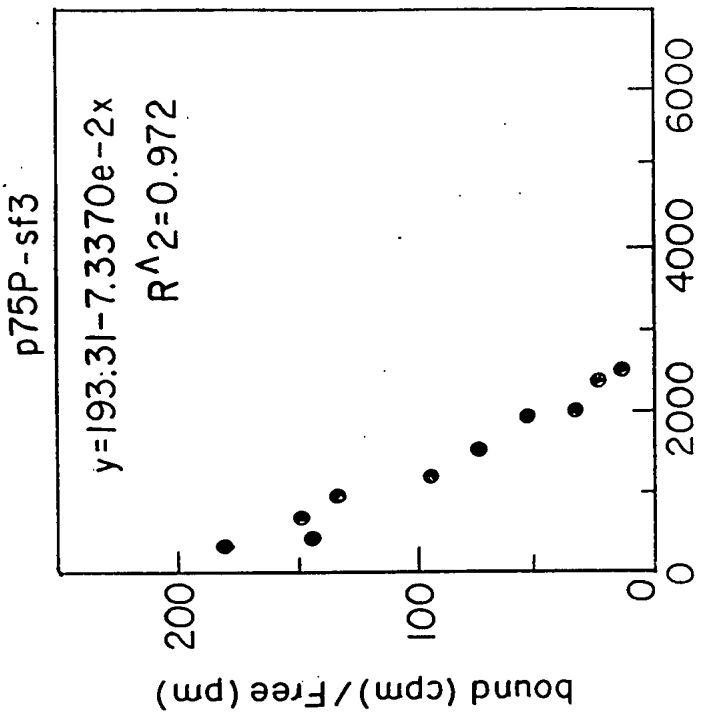


FIG. 33H

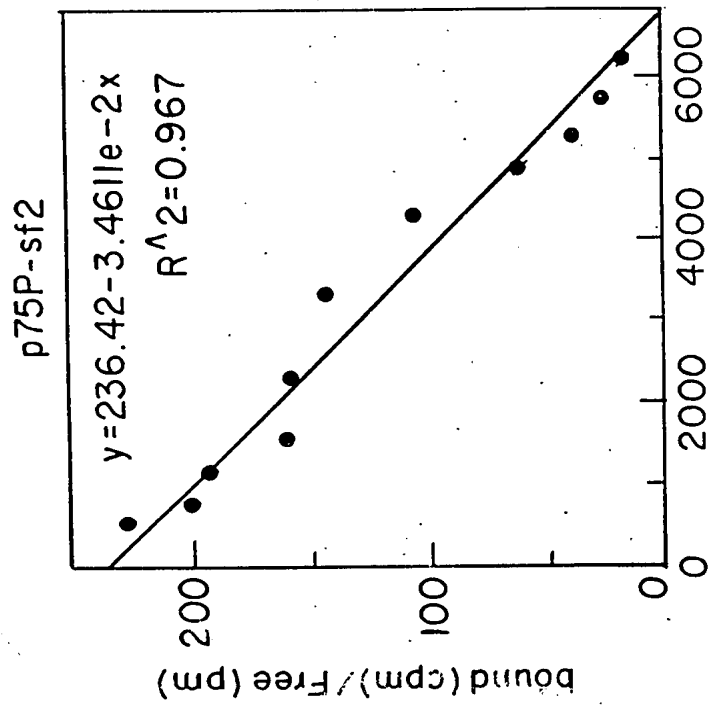


FIG. 33G